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ROBERT LARSSON,
Editor »Hereditas».
Adelgatan 3, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

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X- AND Y-LINKED INHERITANCE IN MELANDRIUM

BY Ö. WINGE
GENETICS LABORATORY OF THE ROYAL VETERINARY AND AGRICULTURAL COLLEGE, COPENHAGEN

I. INTRODUCTION.

THE two only cases in which *X*- and *Y*-linked inheritance has been stated in plants, concern the same species, viz. *Melandrium album*, or its near allied, *M. rubrum*.

In 1923 I demonstrated that a sex chromosome pair, *X* and *Y*, was really present in the *Melandrium* male, which was also found, contemporaneously, by Miss K. BLACKBURN (1923, 1924). In 1925 MEURMAN showed that the larger sex chromosome was the *X*, the smaller one the *Y*, as I had supposed.

BAUR (1911, 1912) and SHULL (1914) found a recessive *X*-linked character, »*angustifolia*», which is lethal to pollen (WINGE 1927 a), and I myself published (WINGE 1927 a) a case of inheritance of »*chlorina*», which I regarded as *Y*-linked, though I discussed different other possibilities. It is obvious that when all observed *chlorina* individuals in *Melandrium* are males in all crossings, there must be some sex-linked inheritance in it, but it is difficult to say, from the experimental results, whether a *chlorina*-gene is linked to the *X*- or to the *Y*-chromosome. Since, however, by backcrossing *chlorina* males with a mother plant, which was able to give *chlorina* offspring, all *chlorina* plants were males, I thought it necessary to conclude that the *chlorina*-gene was definitely located in the *Y*-chromosome. Had it been in the *X*-chromosome, *chlorina* females ought to be segregated, but female *chlorina* plants were never found.

Through my later investigations, which have been very extensive and which comprise 3—4 further generations, I found it necessary, in the case of *chlorina* to alter my opinion and to regard the gene in question as *X*-linked and not *Y*-linked. The reason, why *chlorina* females are never found is simply that the gene is lethal in homozygotic condition; this fact could not be established from the rather scanty material I had during the first generations, but in my later material it is easily seen that the homozygotic individuals are killed. It is

better, therefore, to call the character in question »*aurea*», as it is well known that *aurea* is commonly lethal in double dose. This new interpretation was published in my Danish text-book of genetics, »Arvelighedslære» (Copenhagen 1928) and was set forth in a paper »*X*- and *Y*-linked inheritance in plants», read before the Fifth International Botanical Congress at Cambridge (August 1930).

Besides this gene I have found three others in *Melandrium*, which are inherited in a sex-linked way. One of these is »*abnormal*», which is found in both *X* and *Y*, and another character is »*variegated*», an inhibitor of which is located in the *Y*-chromosome. A third character, which in some cases shows sex-linked inheritance, is »*hermaphroditic males*», but the inheritance of this character is very complicated, as the sex-determination more than other characters depends upon the whole physiological balance, which is decided by the entire sum of genes in the plant.

II. DIFFICULTY OF LOCATING THE PARTICULAR GENES BY COMBINED AUTOSOMAL AND SEX-LINKED INHERITANCE OF INHIBITORS.

It is obvious that when we find a recessive or hypostatic character that is inhibited by different genes, some of which are autosomal, while others are *X*-linked, it is a matter of judgement, whether we are to attribute a special positive gene for that character to the *X*-chromosome, and the inhibitors to the autosomes, or vice versa. Say, we have a variegated type, which is checked by two autosomal genes, *A* and *B*, so that all variegated plants must be *aabb*. Furthermore there is an inhibitor, *C*, in the *X*-chromosome. The variegated female plants, then, must have the formula $aabbX_cX_c$. Under these circumstances it is impossible to say, whether you have a special gene, *c*, for »*variegated*» in the *X*-chromosome, as you could even as well conclude that you have a particular gene for »*variegated*», *a*, in an autosome, and two inhibitors, *B* and *C*, with their recessive allelomorphs, *B* or *b* in an autosome, and *C* or *c* in the *X*. Or you could say that »*variegated*» is produced by the rest-sum of genes in the plant, when the three inhibitors *A*, *B* and *C* are absent. The difficulty is that we are not able to analyze the recessives, *a*, *b*, or *c*, which are possibly nothing but the absence of their dominant allelomorphs.

Objections of this kind prevent me from giving in the following an absolute interpretation of the inheritance of the characters in ques-

tion. Nevertheless, the existence of genes in the sex chromosomes for the characters that are already named is demonstrated with full certainty, and I have formulated the effects of the genes as likely as I could do.

III. THE INHERITANCE OF »*AUREA*».

»*aurea*» (= *au*) is a recessive lethal in the *X*-chromosome.

A is an autosomal inhibitor of *aurea*.

Hence $X_{au}X_{au}$-females are killed, unless they have *A*, in which case they are normally green. All *aurea* plants are males.

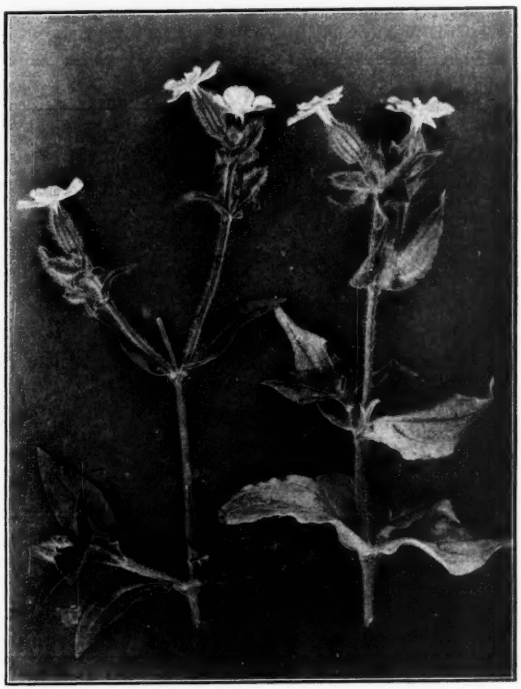


Fig. 1. Left: normal green male, and right: *aurea* male, both from S. 2986.

The *aurea* plants (Fig. 1) are yellowish-green, the leaves being unevenly coloured, and commonly most intensively yellowish at the base of the leaf. The young plants cannot generally be distinguished from the normal ones; but when the plants are on the rosette-stage, or when they are in flower, they can usually be distinguished from the green individuals without any difficulty. The *aurea* plants are somewhat slower growing than the normal, and are therefore more inclined to stop at the rosette-stage without flowering.

Fig. 2 shows the genealogy of some of the material, in which *aurea* plants are segregated. The type originated, as is seen, from a cross between a *Melandrium rubrum* female with a *M. album* male.

All hermaphroditic plants are counted as males, as the cytological analysis of a rather large number of hermaphrodites in my material has shown that they all have an *X*—*Y* chromosome pair at the reduction division (see chapter VI).

Aurea males must always have the formula $aaX_{au}Y$. The mother of an *aurea* male must have one of three formulæ: 1) $AaX_{au}X$, 2) $AaX_{au}X_{au}$ or 3) $aaX_{au}X$. (The *X* without index is supposed to possess the normal dominant allelomorph to *aurea*). By crossing an *aurea* male with its mother we can obtain two types of segregation, viz. 3 : 3 : 1 or 1 : 1 : 1 (see Table 1).

TABLE 1.

	green ♀♀	green ♂♂	*aurea* ♂♂
$AaX_{au}X \times aaX_{au}Y =$	$AaX_{au}X_{au}$ $AaX_{au}X$ (3) $aaX_{au}X$	$AaX_{au}Y$ $AaXY$ (3) $aaXY$	$aaX_{au}Y$ (1)
$AaX_{au}X_{au} \times aaX_{au}Y =$	$AaX_{au}X_{au}$ (1)	$AaX_{au}Y$ (1)	$aaX_{au}Y$ (1)
$aaX_{au}X \times aaX_{au}Y =$	$aaX_{au}X$ (1)	$aaXY$ (1)	$aaX_{au}Y$ (1)

As the two last results are identical, it is sometimes impossible to say which of the two formulæ applies to the mother.

In Fig. 2 the following segregations (Table 2) should be of the first type, 3 : 3 : 1, since the mother, S. 1543—3, was known to have $AaX_{au}X$.

TABLE 2.

	S. 2985	S. 2986	S. 2989	S. 3804	total	theor.	ratio
green ♀♀	8	8	58	126	200	187,7	3
green ♂♂	9	8	50	117	184	187,7	3
aurea ♂♂	1	5	20	28	54	62,6	1
	—	—	—	—	438	438,0	—

Furthermore, S. 2989—5 was supposed to have the same formula as S. 1543—3, and ought to give the same segregation when crossed to an *aurea* male. The agreement is, however, less good, S. 3808 giving 503 : 619 : 366, which is rather far from the 3 : 3 : 1-ratio.

It is to be mentioned that in *Melandrium* and other dioecious plants there is very often, though not always, a surplus of females, owing to

the competition between the male- and the female-determining pollen at the fertilization, as shown by CORRENS (see WINGE 1923 a). As, furthermore, the *aurea* type is, on an average, less viable than the green plants, there is a tendency to find too few *aurea* males and too many green females, relatively.

By inbreeding the material I attempted to eliminate the inhibiting autosomal *A*-gene, but, as already noted, the simplest possible segregation can arise in two ways, and it is a very complicated matter to settle whether, in each case, a female has the formula $AaX_{au}X_{au}$ or $aaX_{au}X$. The reader is invited to meditate upon this problem.

Under all circumstances it is obvious that the green females from a 1 : 1 : 1-segregation always have such a formula ($AaX_{au}X_{au}$ or $aaX_{au}X$) that they, when crossed with an *aurea* male, will themselves give a 1 : 1 : 1-segregation anew.

The segregations put together in Table 3 are all of that category.

TABLE 3. *Crossings of the type* $\left.\begin{matrix} AaX_{au}X_{au} \\ \text{and } aaX_{au}X \end{matrix}\right\} \times aaX_{au}Y$, *giving 1 : 1 : 1-segregations.*

	green ♀♀	green ♂♂	*aurea* ♂♂	total		green ♀♀	green ♂♂	*aurea* ♂♂	total
S. 3806	318	344	306	968	S. 4256	173	198	139	510
S. 3807	211	283	227	721	S. 4257	41	31	47	119
S. 3809	647	670	550	1867	S. 4261	24	33	26	83
S. 3810	520	484	408	1412	S. 4758	185	146	158	489
S. 3811	717	679	518	1914	S. 4759	212	182	193	587
S. 3812	649	566	539	1754	S. 4760	253	205	160	618
S. 4251	354	424	310	1088	S. 4784	74	49	27	150
S. 4252	332	267	268	867	total ...	5823	5538	4586	15947
S. 4253	206	208	217	631					
S. 4254	444	454	194	1092	(theor.)	(5315,7) (1)	(5315,7) (1)	(5315,7) (1)	
S. 4255	463	315	299	1077					

Bearing in mind the relative weakness of the *aurea* males and the fact that not a single *aurea* female has been segregated, there can be no doubt as to the sex-linked inheritance of the lethal *aurea*-gene. The weakness of the *aurea* type is seen from the fact that proportionally many *aurea* plants do not flower the first year and, then, commonly die during the winter.

Some of the segregations in Fig. 2 agree with a 4 : 3 : 1-ratio — one of the ratios to be expected —, as seen in the pedigree. S. 2989—5 ♀

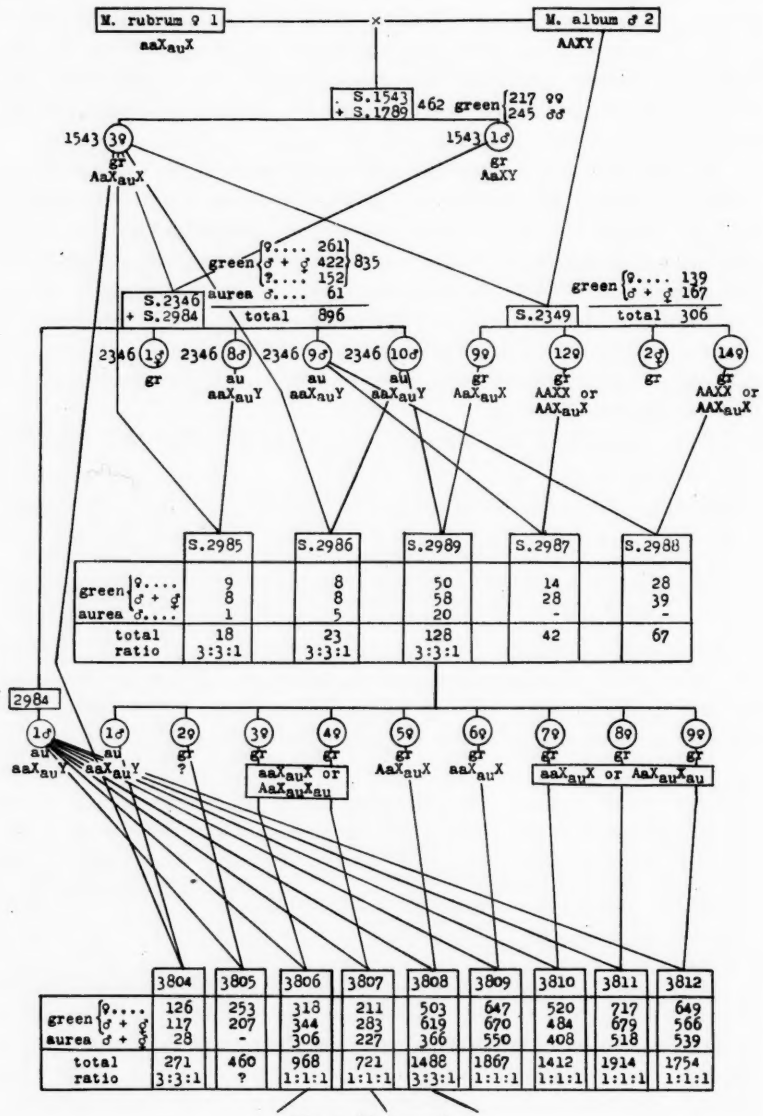
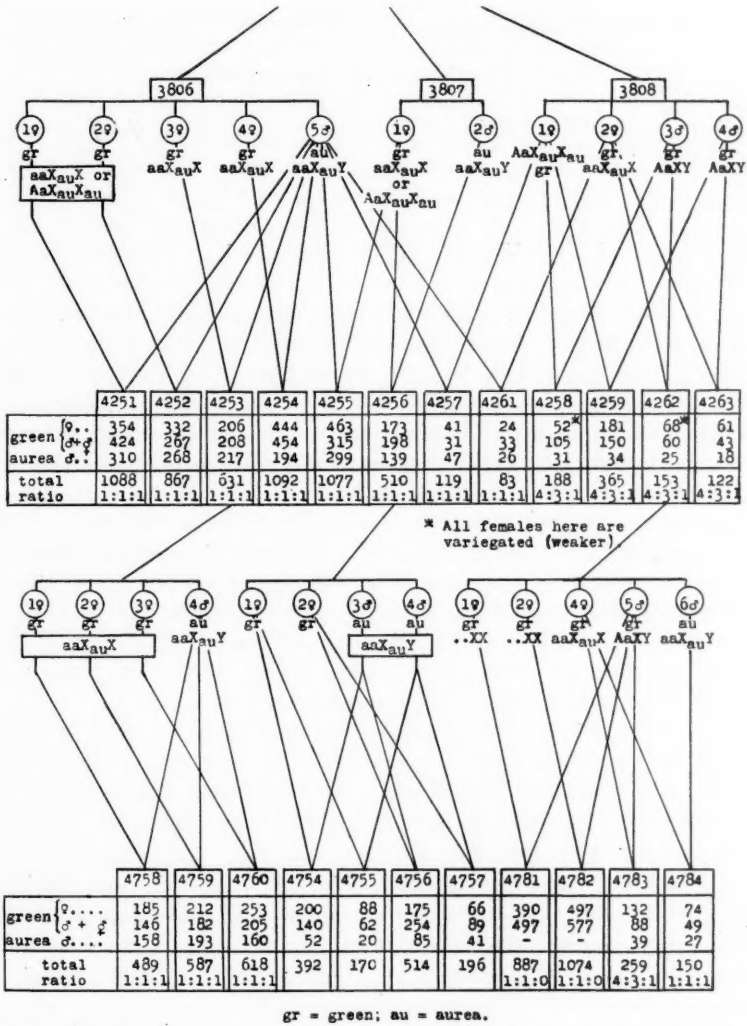


Fig. 2 (first part).

is supposed to have $AaX_{au}X$, wherefore A is present also in some of the individuals of S. 3808 and in the descendants from this sowing.



	4251	4252	4253	4254	4255	4256	4257	4261	4258	4259	4262	4263
green ♀..	354	332	206	444	463	173	41	24	52*	181	68*	61
green ♂+♀	424	267	208	454	315	198	31	33	105	150	60	43
aurea ♂..	310	268	217	194	299	139	47	26	31	34	25	18
total	1088	867	631	1092	1077	510	119	83	188	365	153	122
ratio	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1	4:3:1	4:3:1	4:3:1	4:3:1

	4758	4759	4760	4754	4755	4756	4757	4781	4782	4783	4784
green ♀....	185	212	253	200	88	175	66	390	497	132	74
green ♂+♀	146	182	205	140	62	254	89	497	577	88	49
aurea ♂....	158	193	160	52	20	85	41	-	-	39	27
total	489	587	618	392	170	514	196	887	1074	259	150
ratio	1:1:1	1:1:1	1:1:1					1:1:0	1:1:0	4:3:1	1:1:1

gr = green; au = aurea.

Fig. 2 (continued): Pedigree showing the *X*-linked inheritance of »*aurea*« (*au*).

The 4 : 3 : 1-ratios have arisen through the crossing of *AaXY* males with females of the formula $AaX_{au}X_{au}$ or $aaX_{au}X$.

In Table 4 are collected all the segregations of this category from Fig. 2.

TABLE 4. *Crossings of the type* $\left|\begin{matrix} AaX_{au}X_{au} \text{ or} \\ aaX_{au}X \end{matrix}\right. \times AaX_{au}Y$, *theoretically giving 4 : 3 : 1-segregations.*

	S. 4258	S. 4259	S. 4262	S. 4263	S. 4783	total	(theoret.)
green ♀♀	52	181	68	61	132	494	(4) 543,5
green ♂♂	105	150	60	43	88	446	(3) 407,6
aurea ♂♂	31	34	25	18	39	147	(1) 135,9
total	188	365	153	122	259	1087	1087,0

The numbers of the different types is not very far from the expectation, except in S. 4258, where the females are very few. In this sowing, however, and in S. 4262, the females were not really green, but variegated, owing to genes, which are not further considered in this connection. Since the variegated type is less viable than the normal, this complication will contribute to the obliquity of the segregation.

The correctness of the formulæ is corroborated by the next generation, represented by the sowings S. 4781, S. 4782, S. 4783 and S. 4784, which all descend from the sowing S. 4262. The two first named of those segregated no *aurea* males at all, but gave only green plants, since their mothers had no *aurea-X*, viz. a total of 887 green females and 1074 green males (see Fig. 2). The surplus of males is partly due to the fact that all »green» females were really »variegated» and hence on an average less viable than normal green plants.

As to S. 4783, which segregated *aurea* males, the parent plants must, in accordance with the preceding, have the formulæ $aaX_{au}X$ and $AaXY$, since both were green. The segregation should, therefore, be a 4 : 3 : 1-ratio, and the actual numbers were 132 green females : 88 green males : 39 *aurea* males.

Finally, the fourth sowing, S. 4784, which had the same mother as S. 4783, while the father was an *aurea* male, should theoretically give a 1 : 1 : 1-ratio, since the mother was an $aaX_{au}X$ and the father $aaX_{au}Y$. The actual numbers of offspring were 74 green females : 49 green males : 27 *aurea* males, as seen in Fig. 2.

While the main line is clear, there are, however, three points that I am unable to explain satisfactorily, viz.: 1) the sowing S. 3805; 2) the four sowings S. 4754, S. 4755, S. 4756 and S. 4757, which gave unexpected results (see Fig. 2); and 3) the above-mentioned incongruity in Table 4.

S. 3805 ought to give a 1 : 1 : 1-ratio, but gave only green plants,

253 females and 207 males, in all 460. This sowing was from a cross between a green female, 2989—2, and an *aurea* male, 2984—1. As the parents of the female had the formulæ $AaX_{au}X$ and $aaX_{au}Y$, respectively, their daughters ought to have one X_{au} at least, but as no *aurea* plants were segregated from their daughters something must have happened, and I venture to suggest that a crossing-over between the *X* and the *Y* might have occurred in her father. This supposition is not entirely imaginary, as will be evident from the next chapter, since the *Y*-chromosome in question just contains an *aurea*-inhibitor, which is evidently able to be exchanged between *X* and *Y*, as its presence has been demonstrated in both kinds of sex-chromosomes.

The other question concerns the four mutually related sowings S. 4754—S. 4757, which were expected to give 1 : 1 : 1-segregations, but gave about three times as many green males as *aurea* males, viz. in all: 529 green females; 545 green males; 198 *aurea* males — or 1272 plants together. This seems to be a 3 : 3 : 1-segregation, and not 1 : 1 : 1.

It is certainly possible through the supposition that a mutation has occurred, or, through the hypothesis that a linkage has been broken, to »explain» this exceptional case, but as we have no possibility of checking it, I prefer to abstain from a detailed account concerning this problem. An attempt at making clear the cause of these unexpected results would demand a very extensive work, so much more because we are dealing with a dioecious plant.

About 25000 individuals of *Melandrium* have been counted and sex-determined on analyzing the inheritance of »*aurea*», not taking in consideration the rather large number of plants, which did not flower their first year, and commonly died during the winter at such a large scale that a continued counting next summer was considered worthless.

The main point, however, is that a lethal *aurea*-gene has been located in the *X*-chromosome, killing all $X_{au}X_{au}$ females, unless they possess the autosomal *aurea*-inhibitor *A*.

IV. THE INHERITANCE OF »*ABNORMAL*».

A recessive gene, *n*, which has been located both in the *X*- and in the *Y*-chromosome in *Melandrium*, gives »*abnormal*» plants. The dominant alternative, *N*, inhibits the abnormality, i. e., it gives normal plants. Hence individuals of the formula X_nX_n and X_nY_n are abnormal, while X_NX_N, X_NX_n, X_NY_n, X_nY_N and X_NY_N are normal.

Furthermore there exist, in my material, as many as three auto-

somal inhibitors, *G*, *H* and *I*, which through selection can be discarded from the segregating material, so that very simple segregations will finally result.

The plants, which I have called »*abnormal*» are of a very characteristic appearance (Figs. 3 and 4). The upper part of the plants are of a pale colour, the calyx as well as the stems and the leaves. The calyx never opens, and the petals are entirely hidden, even when the plants are in full flower. »*Abnormal*» plants are less viable than normal; and, furthermore, as we know, male plants are very often, though

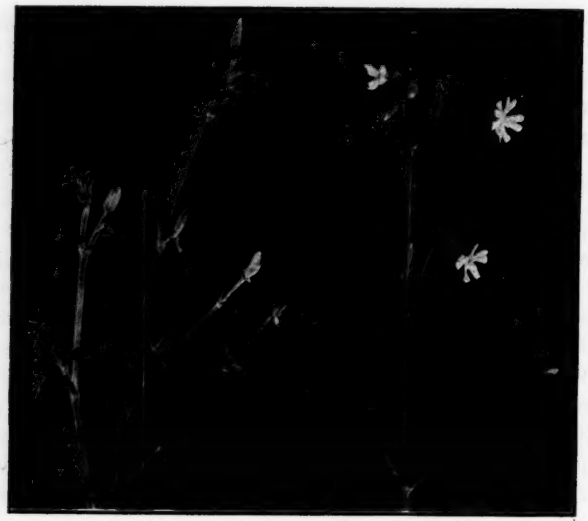


Fig. 3. Left: »*abnormal*», and right: normal female from S. 4788.

not always, in deficit in *Melandrium*. Hence we commonly find that abnormal males are considerably deficient in relation to normal females, when the two types would, theoretically, be segregated in equal numbers.

The »*abnormal*» type was originally segregated from a cross between *Melandrium album* and *M. rubrum*, in the F_2-generation (S. 2983), and appeared also in two backcrosses, S. 2350 and S. 2352, between F_1-plants and a *Melandrium album* female, as shown in Fig. 5.

The F_2-sowing contained 116 normal females, 60 normal males

and 7 abnormal females, while there were no abnormal males. On backcrossing an F_1-male, 1544—1, to the *album*-mother 736 normal females, 353 normal males and 39 abnormal females were segregated in the sowing S. 2352. It was obvious, therefore, that there was a connection between the sex-determination and the character in question.

It was remarkable, however, that in the other backcross, S. 2350, the abnormal plants were all males, since among 1626 individuals 539 were normal females, 1013 normal males and 74 abnormal males, while there was not a single abnormal female.

Fig. 4. Left: »*abnormal*», and right: normal male from S. 4788.

This peculiar difference between the two backcrosses is now entirely cleared up. In the first case, the F_1-male, 1544—1, was from a cross between a *M. album* female and *M. rubrum* male, while in the second case the F_1-male, S. 1543—1, originated from the reciprocal cross, between a *M. rubrum* female and *M. album* male. The distribution of the sex-chromosomes explains fully the different results, as the formulæ in Fig. 5 show.

The experiment was started with five original plants, *M. album* ♀ 1, *album* ♂ 2, *rubrum* ♀ 1, *rubrum* ♂ 2 and *rubrum* ♀ 3. The genetical analysis has shown that these plants had the following formulæ:

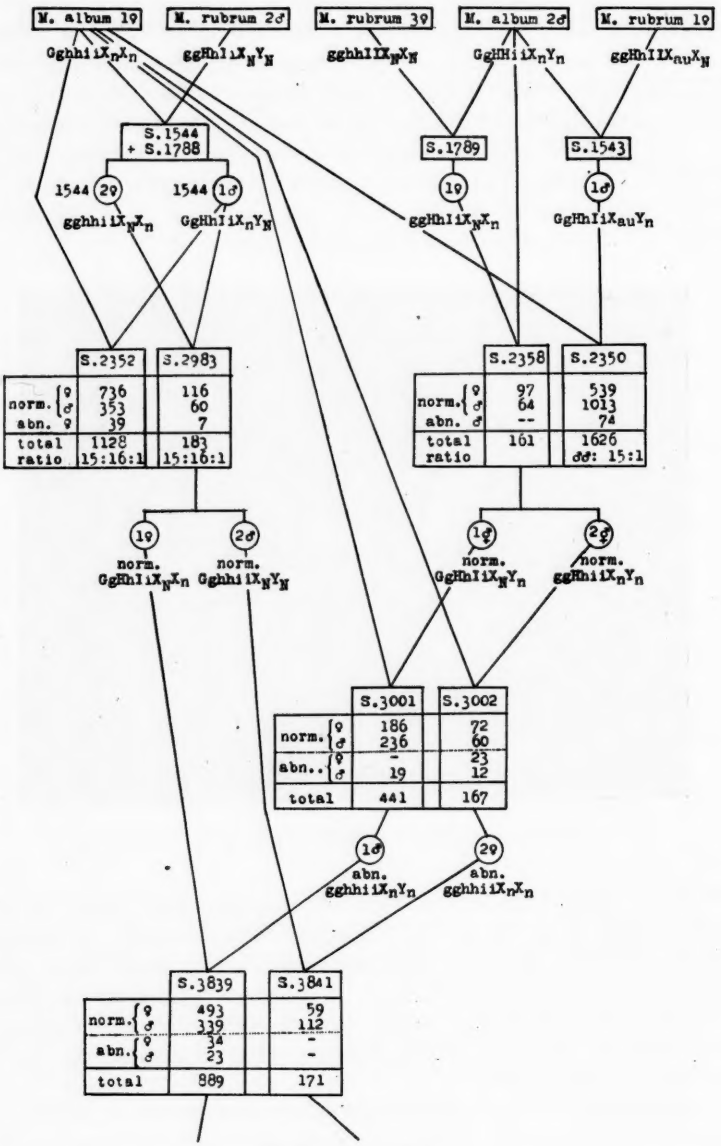
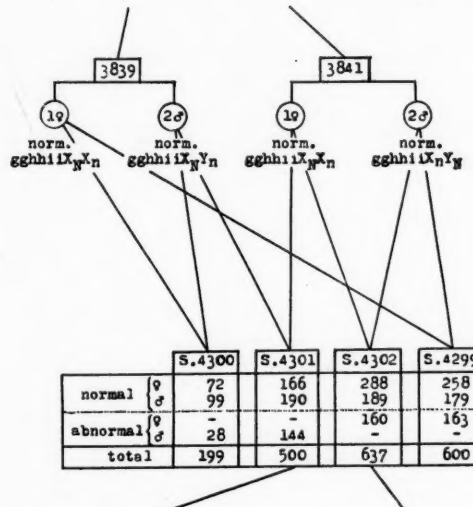
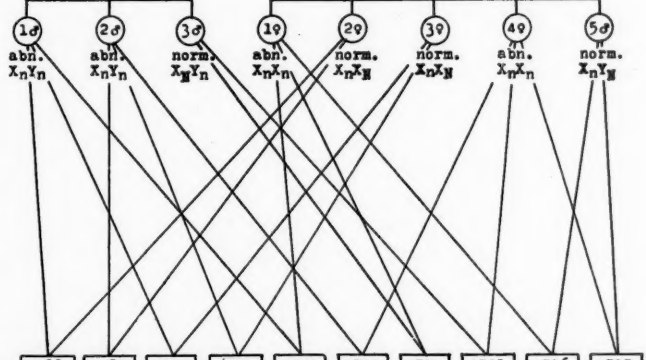


Fig. 5 (first part).



		S.4300	S.4301	S.4302	S.4299
normal	♀	72	166	288	258
	♂	99	190	189	179
abnormal	♀	-	-	160	163
	♂	28	144	-	-
total		199	500	637	600



		4788	4789	4790	4791	4792	4793	4794	4795	4796	4797
norm.	♀	809	537	290	457	-	-	604	160	-	-
	♂	337	228	173	226	-	-	-	-	89	61
abn.	♀	443	338	90	182	148	191	-	-	210	98
	♂	173	151	46	138	52	115	279	62	-	-
total		1762	1254	599	1003	200	306	883	222	299	159
normal		1394	886	525	747	-	-	813	197	141	123
abnormal		860	602	223	395	371	423	458	164	268	180
total		2254	1488	748	1142	371	423	1271	361	409	303

norm. = normal; abn. = abnormal; ♂♂, when present, included in ♂♂.

Fig. 5 (continued): Pedigree showing the *X*- and *Y*-linked inheritance of ›*abnormal*‹ (*n*).

album ♀ 1	*album* ♂ 2	
$GghhiiX_nX_n$	$GgHHiiX_nY_n$	
rubrum ♀ 1	*rubrum* ♂ 2	*rubrum* ♀ 3
$ggHhIIX_{au}X_N$	$ggHhIiX_NY_N$	$gghhIIX_NX_N$

G, *H*, *I* and *N* are inhibitors, while *n* is the »*abnormal*»-gene and *au* the above mentioned »*aurea*»-gene.

There is a certain system in the distribution of the different genes on the two pure species. The two *album* plants had the »*abnormal*»-gene in all *X*'es and in *Y*, while there was no *n* in *rubrum*; the *rubrum* plants had *N* in all sex-chromosomes, except the *rubrum* ♀ 1, which had an *X* with the »*aurea*»-gene as already mentioned above. This gene or, rather, the »*aurea*»-containing *X*-chromosome inhibits the »*abnormal*» character just as *N* does, since I have never obtained abnormal *aurea* plants. As to the autosomal inhibitors, both *album* plants must be supposed to have *ii*, and all *rubrum* plants *gg*; but it is, of course, possible that other individuals of the two species would show the dominant alternatives, *I* and *G*, respectively.

As will be seen in Fig. 5, the above formulæ agree fully with the segregations through all generations, especially as to the different types, which were segregated, while the proportion between the numbers of male and female plants has very often got displaced, as usually in *Melandrium*. In checking the agreement between formulæ and actual segregations the chief point is, therefore, to compare the relation between normal and abnormal plants within the males and within the females respectively, while it is obvious that the total segregations will commonly show obliquities.

In the following Table 5 I have collected the segregations, in which $^1/_{16}$ of the males or of the females or plants of both sexes should theoretically be abnormal (compare with Fig. 5).

The correspondance is seen to be good.

A segregation, in which $^1/_4$ of both sexes should theoretically be abnormal is found in the sowing S. 3002 (see Table 6).

Through inbreeding, and especially through backcrossing to abnormal plants, very plain segregations are obtained, the inhibitors being gradually rejected. When the autosomals are all recessive they can entirely be omitted from the formulæ, and we need only consider the *X*- and *Y*-linked genes.

The earliest 1 : 1-segregations within a single sex, which I have

found, were in 1928, as the result of the following diallel crossing the year before of four plants, two males and two females, all of which were normal (see Fig. 5). This diallel crossing is presented in Table 7.

TABLE 5.

		S. 2352 $GGhhiiX_nX_n \times GgHhIiX_nY_N$	S. 2983 $ggHhiiX_NX_n \times GgIIhIiX_nY_N$	S. 2350 $GghhiiX_nX_n \times GgHhIiX_{an}Y_n$	S. 3001 $GghhiiX_nX_n \times GgHhIiX_NY_n$	S. 3839 $GgHhIiX_NX_n \times gghhiiX_nY_n$
normal	♀♀	736 (15)	116 (15)	539	186	493 (15)
	♂♂	353	60	1013 (15)	236 (15)	339 (15)
ab-normal	♀♀	39 (1)	7 (1)	—	—	34 (1)
	♂♂	—	—	74 (1)	19 (1)	23 (1)
Total		1128	183	1626	441	889
normal to ab-normal; theor. among	♀♀	726,6 : 48,4	115,3 : 7,7			494,1 : 32,9
	♂♂			1019,1 : 67,9	239,1 : 15,9	339,4 : 22,6

TABLE 6.

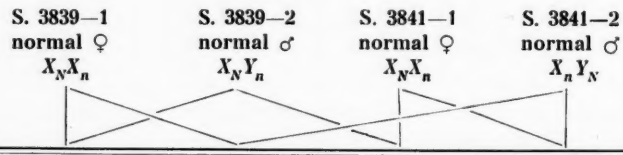
		S. 3002 $GghhiiX_nX_n \times ggHhiiX_nY_n$	
normal	♀♀	72	Theoretically among females: 71,25 : 23,75 males: 54,0 : 18,0
	♂♂	60	
abnormal	♀♀	23	
	♂♂	12	
total		167	

The obliquity in the theoretical 1 : 1-proportions between normal and abnormal within one sex is very conspicuous, indeed, owing to the different viability of the types, but the combined *X*- and *Y*-linked inheritance is doubtless.

As shown in Fig. 5, eight individuals from the two sowings, S. 4301 and S. 4302, were tried for a next generation, viz. two normal males,

two normal females, two abnormal males and two abnormal females. They were crossed in 1928 to each other, diallelic, in ten different combinations, and the results were all exactly in accordance with the expectation (see Fig. 5). It is apparent that the ten combinations contain four types of crossings, giving the following total result (see Table 8).

TABLE 7.



		S. 4300	S. 4299	S. 4301	S. 4302
normal	♀♀	72 $X_N X_N + X_N X_n$	258 $X_N X_n$	166 $X_N X_N + X_N X_n$	288 $X_N X_n$
	♂♂	99 $X_N Y_n$	179 $X_N Y_N + X_n Y_N$	190 $X_N Y_n$	189 $X_N Y_N + X_n Y_N$
abnormal	♀♀	—	163 $X_n X_n$	—	160 $X_n X_n$
	♂♂	28 $X_n Y_n$	—	140 $X_n Y_n$	—
total		199	600	496	637
Inclusive rosettes, which could not be sex-determined	normal	215	550	417	537 (ratio) (3)
	abnormal	69	192	198	171 (1)

Altogether are, as will be seen, 13382 flowering *Melandrium*-individuals classified as normal or abnormal in F_2 and later generations in order to solve the problem concerning the inheritance of »*abnormal*».

The most peculiar fact, I think, is not that genes are demonstrated in the *X*- and *Y*-chromosomes, but that the same pair of alternatives, *N* and *n*, is located in both *X* and *Y*. It makes no particular difference, whether the above formulation of the gene effects is adopted or, possibly, replaced by an other. But, I do not see any possibility of eluding the acceptance of identical genes in *X* and *Y*.

As we know, the difference in size and form of *X* and *Y* in *Melandrium* is very pronounced, the *X* being about twice as large as the *Y*. The demonstration of identical genes in both chromosomes and

TABLE 8.

		normal		abnormal		total
		♀♀	♂♂	♀♀	♂♂	
S. 4792 ..... S. 4793	$X_nX_n \times X_nY_n$	—	—	339	167	506
S. 4788 ..... S. 4789 ..... S. 4790 ..... S. 4791	$X_NX_n \times X_nY_n$	2093	964	1053	508	4618
S. 4794 ..... S. 4795	$X_nX_n \times X_NY_n$	764	—	—	341	1105
S. 4796 ..... S. 4797	$X_nX_n \times X_nY_N$	—	147	308	—	455
					grand total	6684

in the same material suggests that crossing-over between *X* and *Y* is possible, but the details of this scope fall outside the scope of this paper. On demonstrating the *X*—*Y*-crossing-over in *Lebistes* (1922, 1923 b, 1927 b) I just pointed out that in this species the agreement as to the shape of the two sex chromosomes was probably a necessary condition. In this connection, however, I wish to call to mind that at least by *Humulus* the *Y*-chromosome is evidently composed of two *X*'es (WINGE 1929). It might be possible that in *Melandrium* the sex chromosomes did arise in a similar way, from autosomes, and that the large *X* in this plant originally was composed of two *Y*'s, and that the *X*-chromosome even now is able to exchange one of its elements, which is then replaced by the *Y*.

V. THE INHERITANCE OF »*VARIEGATED*».

Three autosomal genes, *L*, *M* and *O*, inhibit »*variegated*», but as also the *Y*-chromosome, normal (Y_N) as well as »*abnormal*» (Y_n) (see above), inhibits this character, the variegated plants are always females. In the different *X*'es I have found no inhibitors.

llmmooXX-plants are all variegated, therefore, while *llmmooXY*-individuals are green. It should be mentioned, however, that it is possible in some cases to demonstrate a very weak variegation in the males also, but this is an exception, and Fig. 6 shows to the left one of the most variegated leaves, which could be found in a male, and, to

the right, three ordinary leaves from variegated females. The variegation will commonly be seen on the rosette-stage already. Some variation is found as to the intensity of the variegation, but there is a gap between the least variegated females and the most variegated males, which as a rule makes the classification easy.

In segregations where all females should, theoretically, be variegated, I have only once found two females which were apparently entirely green. On trying them for a next generation they

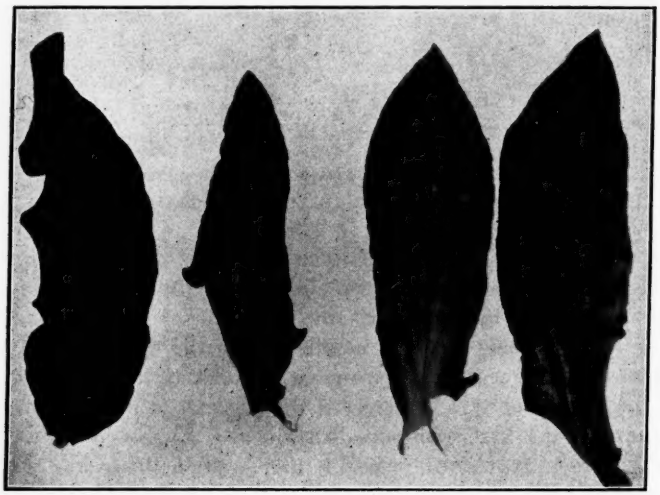


Fig. 6. Leaves of S. 4784 showing the type of constant variegated females and non-variegated or faintly variegated males (see text). The leaf most to the left is of a male, the other three leaves are from females.

were shown to agree genetically with the variegated type (see Fig. 7, S. 4262—1 and S. 4262—2).

The first variegated plants were found among descendants from the same cross as gave *aurea* plants, i. e., from a cross between *M. rubrum* ♀ 1 and *M. album* ♂ 2, in the fourth generation, namely in the sowings S. 3807, S. 3808 and S. 3809 (see Fig. 2). In those sowings half of the females were killed owing to homozygosity as to the *aurea*-gene, as already mentioned. The following numbers of variegated were found in this early material [1] (see Table 9).

[1] By comparing with Fig. 2 it will be seen that the classification of the segregated types in the pedigree is not identical with the present one. This is simply

Out of 1361 females, therefore, 220 were variegated, while the males were non-variegated. It is true that at the very beginning of the investigation, when all plants with even the slightest degree of variegation were counted as variegated, 14 males among a total of 2715 were counted in this group. This very weak variegation in the males, however, is evidently owing to the *Y*-inhibitor not being perfectly epistatic in all those males which are entirely recessive, so that *llmmooXY*-males by an exception will show a very weak variegation. As already pointed out there is, however, usually a very distinct gap between those males and the really variegated females.

TABLE 9.

	S. 3807	S. 3808	S. 3809	Total
non-variegated ♀♀.....	171	411	559	1141
variegated ♀♀.....	40	92	88	220
non-variegated ♂♂.....	510	985	1220	2715
total	721	1488	1867	4076

The analysis of the mode of inheritance of »*variegated*» is presented in Fig. 7, starting with the variegated female S. 3808—2 and the brother S. 3808—3, which was selected as the most (though only slightly) variegated male. The same female was furthermore crossed to a green normal male from the »*abnormal*» line S. 3841—2.

The contents of the *X*'es and the *Y*'s is shown in the pedigree, S. 3808—2 having $X_{au}X_n$ and being homozygous as to the »*abnormal*»-inhibitor *G*. The brother, S. 3808—3, has X_nY_n (compare with Fig. 2, where the presence of the »*abnormal*»-gene, however, is not indicated), but the »*abnormal*»-character is inhibited by *G*, which is present in double dose. The second male, S. 3841—2, has the formula $AaggLIMMOOX_nY_N$ (compare with Fig. 5), being heterozygous, there-

because, in the pedigree, the variegated plants were not counted separately, but were added to the green ones. On the other hand, the *aurea* males that were counted separately in the pedigree are counted together with the green ones here. As to the hermaphrodites they are counted as males, as they have an *X*- and a *Y*-chromosome. In order to obtain a practical survey it is necessary to leave out of consideration in every case all characters that are not the objects of the actual investigation. On the other hand it is just the advantage of the present investigations that so many diallel crossings have been made, and so many genes in the *X*'es and *Y*'s are known that one has an excellent test of the validity of the whole theoretical structure.

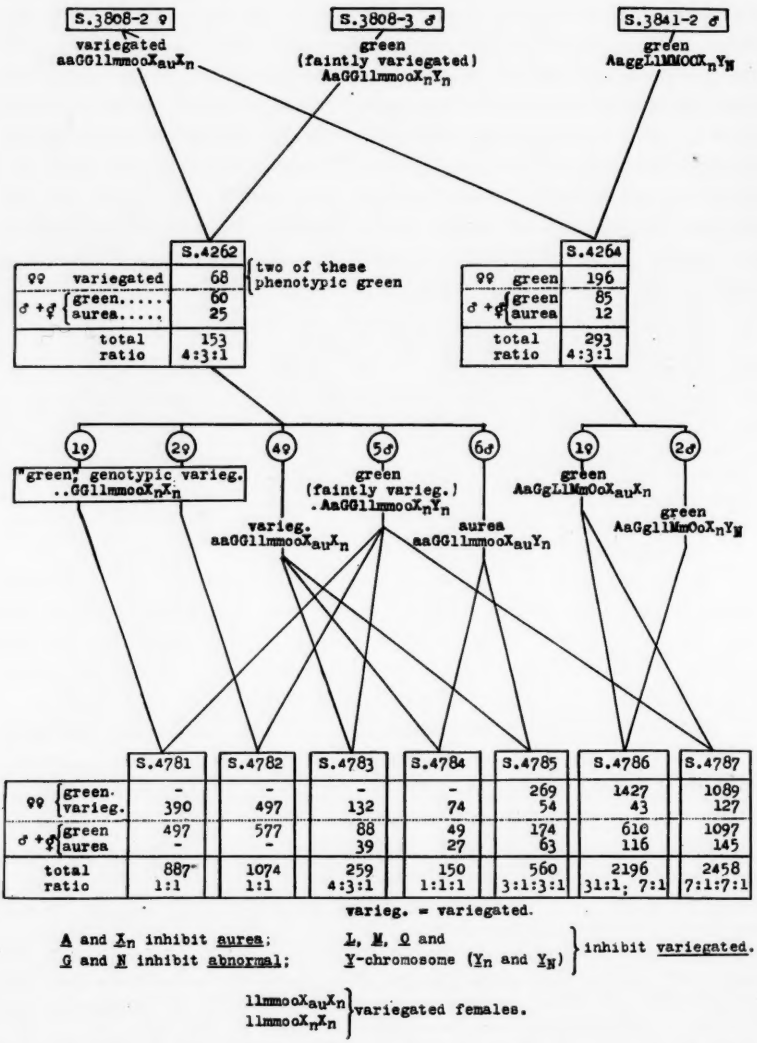


Fig. 7. Pedigree showing the inhibiting effect of the *Y*-chromosome on variegation.

fore, as to the autosomal »*aurea*»-inhibitor, *A*, recessive as to the »*abnormal*»-inhibitor, *G*, and furthermore having three autosomal inhibitors for variegation, *L*, *M* and *O*, two of which are present in double

CORRIGENDUM.

On Fig. 7, page 146, the father of S. 4785 was erroneously given as S. 4262—6, while it should be S. 4264—2.

dose, while it is heterozygous as to the third. Finally it has the recessive »*abnormal*»-gene, *n*, in the *X*, and the normal dominant, *N*, in the *Y*. As will be seen from the pedigree, Fig. 7, the formulæ are all in conformity with the origin of the plants. The methods of diallel crossings, which I am always using in genetic experiments, give a very effective proof as to the correctness of the formulæ, especially as to the sex chromosomes. The segregations, presented in Fig. 7 are in full accordance with the types, which are segregated, while the proportion between the numbers of the individuals in the different groups is very often modified by the different viability of the types and by the obliquity in the sex-percentage.

The variegated S. 3808—2 ♀, $aaGGX_{au}X_n$, crossed with the brother, S. 3808—3 ♂, $AaGGX_nY_n$, should theoretically segregate 4 variegated ♀♀ : 3 green ♂ : 1 *aurea* ♂, and gave 68 : 60 : 25 respectively. All females are genetically variegated, because the inhibitors are eliminated. In this material, however, appeared, besides 66 variegated, the two above named apparently green females, S. 4262—1 and S. 4262—2. They were tested for the next generation and were found to be genetically identical with the variegated, for which reason they have been counted to that group. The real cause of their different appearance has not been cleared up, but may be suggested to be due to an accidental accumulation of minor genes influencing the leaf colour, but being unable to disturb the principal rule of heredity. No »*abnormal*» are segregated owing to the inhibitor *G*.

On crossing the same female, S. 3808—2, with S. 3841—2 ♂, AaX_nY_N, the theoretical segregation must be 4 green ♀♀ : 3 green ♂ : 1 *aurea* ♂. The counts gave 196 : 85 : 12. No variegated are segregated, since the father is homozygous as to two inhibitors, *M* and *O*, and no abnormal are found, because the mother had *GG*.

The next generation was obtained through diallel crossing of types from the two families, as Fig. 7 shows. As will be seen two sowings, S. 4781 and S. 4782, gave only green males and variegated females, 1074 ♂♂ and 887 ♀♀ together. Two sowings, S. 4783 and S. 4784, segregated *aurea* males in addition. S. 4785 and S. 4787 segregated four types, green females, variegated females, green males and *aurea* males. Finally, S. 4786 gave a very complicated segregation, as the parents had the following formulæ respectively: $AaGgLlMmOoX_{au}X_n$ and $AaGgllMmOoX_nY_N$, so the segregation should theoretically be according with a 217 : 7 : 224 : 32 : 31 : 1-scheme. The actual numbers were as follows (Table 10).

TABLE 10.

		actually found	theoret. among	
			♀♀	♂♂
normal	green ♀♀	1319	1246,0	
	variegated ♀♀	39	40,2	
	green ♂♂	610		544,5
	aurea ♂♂	116		181,5
abnormal	green ♀♀	108	178,0	
	variegated ♀♀	4	5,7	

The surplus of females, 1470 against 726 males, is very considerable. The deficiency of abnormal plants and of *aurea* individuals must be due to lesser viability. In accordance with the theory all variegated are females, all *aurea* are males, and all abnormal are females.

As to S. 4787 this is the result of back-crossing an F_1 female, S. 4264—1 ($AaGgLlMmOoX_{au}X_n$) to a recessive, faintly variegated male, S. 4262—5 ($AaGGllmmooX_nY_n$) (see Table 11).

TABLE 11.

		found	theror.	ratio
♀♀	green	1089	1075,4	7
	variegated	127	153,6	1
♂♂	green	1097	1075,4	7
	aurea	145	153,6	1

This generation comprises 7584 flowering plants in all, and it will be seen that the types which are segregated in all cases agree with the formulæ of the parents and with the earlier generations.

VI. THE INHERITANCE OF »*HERMAPHRODITIC MALES*».

Hermaphrodites appeared in the F_2-generation after crossing *Melandrium rubrum* ♀ 1 with *M. album* ♂ 2 (see Fig. 2). The F_1-generation contained 217 ♀♀, 245 ♂♂ and no ⚥, but on crossing two

F_1-individuals an F_2-generation arised, in which were counted 261 ♀♀, 407 ♂♂ and 59 ♂♀♀. It might be of some interest to find out the genetics of these hermaphrodites.

The inheritance of hermaphroditism in *Melandrium* has formerly been experimentally analyzed by SHULL (1910, 1911) and later by G. and P. HERTWIG (1922). SHULL found two types of hermaphrodites, »genetic» and »somatic». From his summary (1911) I am quoting the following:

»When the genetic hermaphrodites are used as pollen parents, either when self-fertilized or in crosses with females, their progenies consist of females and

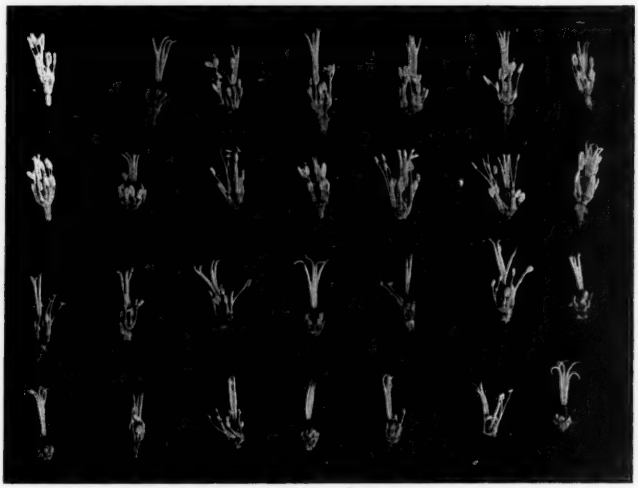


Fig. 8. Flowers from segregated hermaphroditic plants; sepals and petals are removed. The left flower in the upper row is from a pure male.

hermaphrodites. When they are used as pistil parents, and fertilized by normal males, they produce females and normal males».

»The fact that males can be modified so as to produce functional organs of both sexes, indicates that they are sex heterozygotes, and the production of both females and hermaphrodites by self-fertilized hermaphrodites strongly supports the same interpretation».

»Somatic hermaphrodites may be externally indistinguishable from the genetic hermaphrodites, but when used as pollen parents they produce no hermaphrodite offspring, but only females and normal males».

»The hermaphrodite character can neither find expression in the females, nor can it be transmitted by their eggs to the male offspring».

»Among the offspring of genetic hermaphrodites were a small number of male mutants (11 in 5467), which on breeding proved to be normal males».

»The view is expressed that the sexes represent alternative states which in different species may be attained in various ways, through either quantitative or qualitative changes, additions, subtractions, substitutions, or transformations, and that in some instances the action of environment may prove effective in determining which of these states shall find expression».

In some of these points I am in full accordance with SHULL, while in others I can not agree with him.

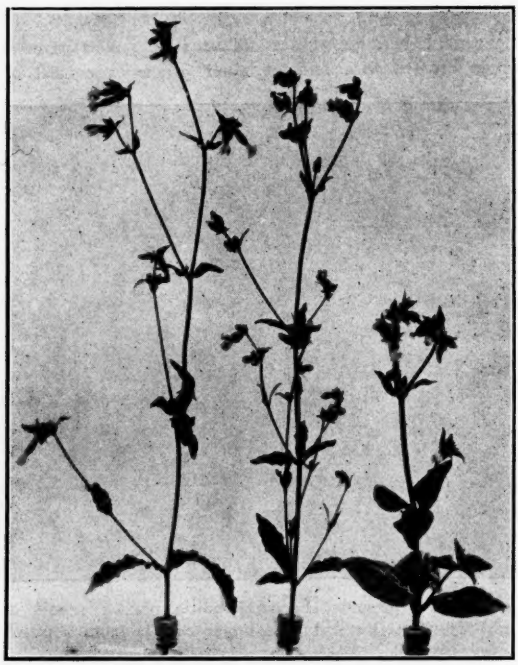


Fig. 9. A pure male (left) and two hermaphroditic plants showing the verticillate leaves.

As to the nature of the hermaphrodites, they are, in my material, in accordance with SHULL, apparently all modified males, since all individuals, which have been cytologically analyzed (ab. 15 in number) had an *X*- and a *Y*-chromosome, as it is normally found in males. They agree, therefore, with the *Melandrium* hermaphrodites investigated by BĚLAŘ (1925), while *XX*-hermaphrodites as described by ÅKERLUND (1927) were never found by me. Even the most female hermaphrodites with very short stamens were *X*—*Y*-individuals. In

the foregoing pages, therefore, the hermaphrodites have been counted as males in order not to complicate the matter.

On Fig. 8 are photographed one male and a series of hermaphroditic flowers whose sepals and petals have been removed. The length of the stamens and the size of the pistils and the ovaries is seen to vary considerably.

The hermaphrodites have a characteristic appearance, which makes it possible, as a rule, to identify the type at some distance. Rather often they have, in my material at least, verticillate leaves, viz.

Fig. 10. Three hermaphroditic plants showing fasciation of stems.

three (or four) at the same level. Some of them are nearly sterile, while others set small capsules, containing seeds of a more brownish than bluish colour, while the seeds from female plants are larger and bluish. On Fig. 9 are seen three plants, two of which (at right) are hermaphroditic males, while the third (at left) is a normal male. Some of the hermaphroditic plants are more or less fasciated, as shown on Fig 10.

The view of SHULL, however, of the existence of two distinct types of hermaphroditic males, »genetic» and »somatic», is not in agreement with my experiments. It is true that on crossing a hermaphroditic male with a normal female sometimes only male and female

offspring and no hermaphrodites are segregated, but on intercrossing the offspring hermaphrodites are segregated in the next generation again. The character in question has simply been inhibited in one generation as other recessive or hypostatic characters. SHULL, however, also tried a second generation in order to verify that »there was not simply the temporary disappearance of the hermaphrodite character through some thinkable vagary of dominance in the F_1», but on doing this, he crosses the males to females of an other origin and not to their female sibs.

SHULL found (1911, p. 352) that even full sibs of hermaphrodites were sometimes unlike in their nature, some of them being »genetic» and some »somatic»; but as the different hermaphrodites were crossed to different females, it is impossible to decide, whether they were identical or not. So far as I can see there is, in SHULL's material, only one case, in which the same female, 08109 (1), has been fertilized by both »genetic» and »somatic» hermaphrodites, giving females + hermaphrodites and females + males respectively, but in this case the two types of hermaphrodites were not sibs, but of different origin. As the sex is a question of balance it is undoubtedly necessary to inbreed the material and make diallel crossings in order to analyze the nature of the plants; but, it is of course easier to argue on this matter now twenty years after SHULL's publication, and it must be granted that even now, after we have some better understanding of the sex determination, it is impossible to survey all details as to the hermaphroditism in *Melandrium*. It remains to be said, however, that SHULL's view on the sex determination as expressed in the last sentence, which I have cited, must be regarded as correct even today.

SHULL's statement that the hermaphroditic character can not be transmitted by their eggs is not in agreement with my experimental results, as will be seen later, nor with G. and P. HERTWIG's results (1922). These authors explain their observations on the basis of GOLDSCHMIDT's demonstration of a different strength of genes pulling in male and female direction, and so far I can fully agree with their theoretical considerations. But, I cannot subscribe to their hypothesis, that the hermaphrodites arise through a mutation of two genes at one time, *F* and *f*. From a purely theoretical viewpoint a double-mutation is improbable, and, secondly, a single mutation should suffice for realizing the hermaphroditic type. On the whole I do not think that we need to take the origin of the sex-genes with different strength in consideration. On starting our experiments with a number of plants

there exists beforehand a variation as to the strength of the different sex-genes, and the hermaphroditic types are simply segregated. Only in exceptional instances is it necessary to resort to the acceptance of new mutations that have occurred during the experiments.

My experiments have clearly shown that sex-determining genes are present in the *X*- and the *Y*-chromosomes, as might be expected, and that the hermaphrodites owe their origin to a disturbed balance between genes pulling in male and female direction. These genes, however, are not only present in the sex-chromosomes, but also in the autosomes, and the mode of inheritance of hermaphroditism is therefore very complicated in some cases, while, on the other hand, it is possible to select individuals, in which the inheritance of hermaphroditism is simple, because the autosomal genes have been eliminated, or, rather, because the plants have been homozygous as to autosomal genes, or nearly so.

I shall not attempt to set up the whole scheme with formulæ for all plants which I have used for the analysis, as rather many of the formulæ cannot be controlled properly, especially in the early material, and because the material during the past years has been very extensive and difficult to grasp in all its bearings. The acquaintance with the *X*- and *Y*-linked genes quoted above has been helpful in this experimental work, but it will doubtless be understood that the analysis of hermaphroditism, combined with the inheritance of lethals (as *aurea*), abnormal, variegated, and complicated by obliquity as to the sex-relation and different viability of the types, is a rather difficult task, especially because the question of sex is a question of balance between many genes, some of which are pulling in opposite direction of the others. In the pure species, *Melandrium album* and *M. rubrum*, the *X*- and the *Y*-chromosomes are the only chromosomes that contain genes which are epistatic factors in the determination of the sex, the autosomal sex-genes being homozygously present in a hypostatic condition. By crossing the two species, however, the balance will be disturbed in some of the F_2-plants and in their descendants. Only after some generations it is possible to obtain stability again, especially through inbreeding. But even after stability has been obtained as to the sex-determination, confusion will very often arise anew, when the inbred stable lines are intercrossed, owing to new heterozygosity as to sex-determining genes. This is the explanation, why the inheritance of sex in *Melandrium* is more complicated than the inheritance of most other characters.

I shall prefer to quote only some of the most characteristic facts, and to illustrate the same by giving some of the pedigrees in my material. It needs perhaps not be remarked that when some of the sowings or experiments have been left out of consideration, it is because they were rather unsuccessful or less interesting, but not because they were in conflict with the results which are here set forth.

Hermaphrodites are males, whose *X* (in the given total gene combination) is strongly female in relation to the *Y*, which is relatively

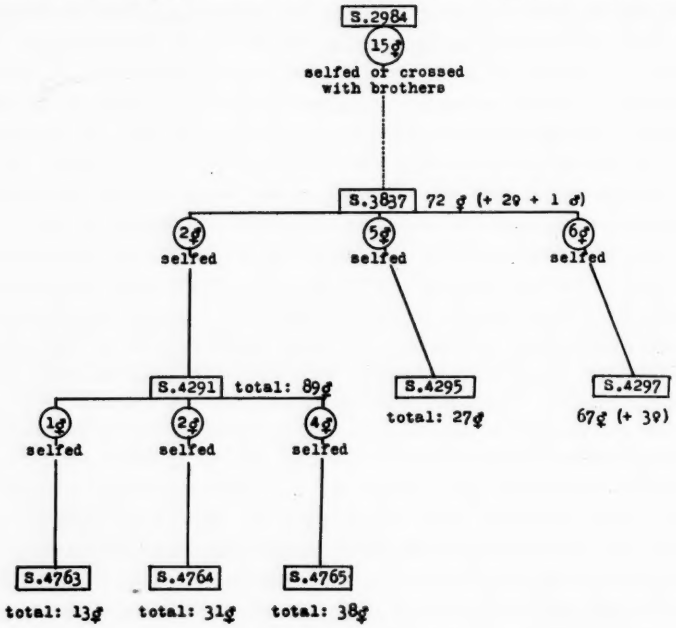


Fig. 11.

weakly male. *XY*-individuals of this type are not males but even as well females, i. e. hermaphrodites. If the strongly female *X* is called X_f, the hermaphroditic formula is X_fY.

C is an autosomal gene, pulling in male direction and hence acting as an inhibitor of hermaphroditism; this gene is present in a great deal of my material.

Constant hermaphrodites. — The simplest case is found, when hermaphrodites are selfed. One of the 59 hermaphrodites in F_2,

S. 2984—15, whose pollination was not controlled, gave 1 ♂, 2 ♀♀ and 72 ⚥⚥. No doubt it has been self-pollinated or crossed with its brothers. Through selfing some of the 72 ⚥⚥ constant hermaphroditic pedigrees were immediately obtained. The genealogical tree is shown in Fig. 11.

It is apparent from Fig. 11 that constancy is obtained, at least in most of the material. Nevertheless there is an exception as to S. 4297, where 3 ♀♀ appeared together with 67 ⚥⚥. Probably these females owe their origin to an accumulation of autosomal genes acting upon the sex determination, the material being not entirely homozygous.

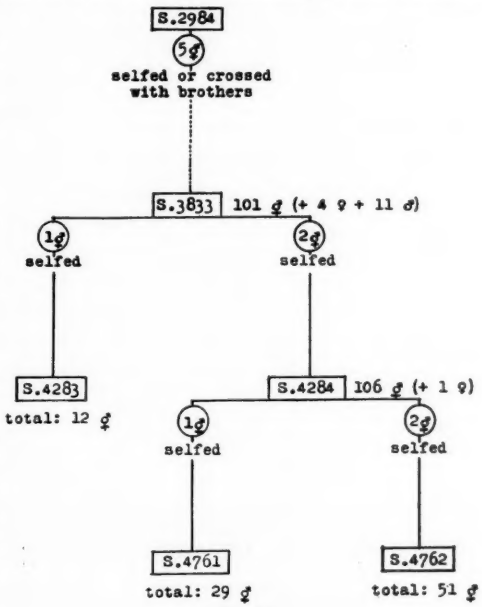


Fig. 12.

The explanation of the types being constantly hermaphroditic is, I think, the following: The constant hermaphrodite is an X_fY-type with a lethal gene in the X_f-chromosome, killing all XX-individuals. It is possible that this lethal is identical with the *aurea*-gene in the X, since S. 2984 contained »*aurea*» (see Fig. 2), so that, in this material the X_{au}-chromosome is strongly female in relation to the Y, which is relatively weakly male, but on the other hand it seems unlikely that this lethal is identical with »*aurea*», since the hermaphrodites were not *aurea*-coloured, and since an inhibitor of *aurea*, if present, should probably prevent the homozygous females from being lethal. Hence I prefer to call this lethal *l*.

The hermaphrodite $X_{f,l}Y$, when selfed, should theoretically segregate

$$\underset{\text{(dies)}}{1\ X_{f,l}X_{f,l}} : \underset{\text{(dies)}}{2\ X_{f,l}Y} : 1\ YY$$

As the females are killed owing to homozygosity as to *l*, and the *YY*-

individuals, as in other organisms, are not viable, all descendants will be $X_f, {}_lY$, i. e. hermaphrodites. It is a balanced lethal type.

Another hermaphrodite, S. 2984—5, which had the same parents as S. 2984—15 in Fig. 11, showed the same inheritance. This plant also was pollinated without control and gave 4 ♀♀, 11 ♂♂ and 101 ⚥⚥. On selfing two of the hermaphrodites, constant hermaphroditism was obtained as shown in Fig. 12.

There is only one plant, which is an exceptional female, viz. in

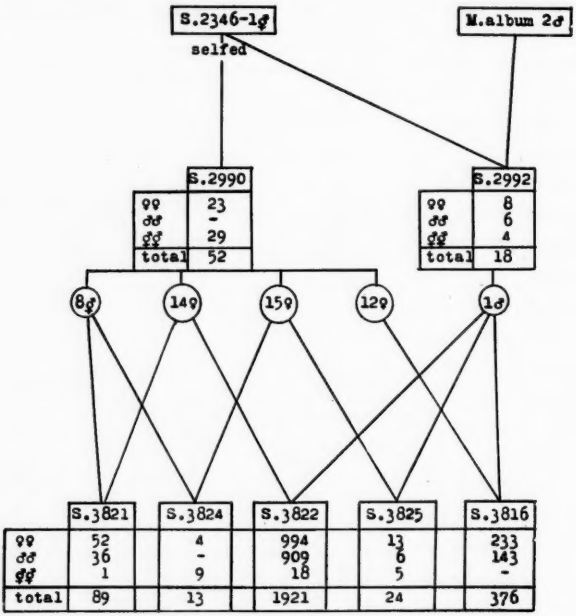


Fig. 13.

S. 4284, all others being hermaphrodites. The occurrence of such deviating individuals in the early generations is already quoted.

It shall be noticed here that the material gained from selfing hermaphrodites gets rather weak through the inbreeding. The seeds, therefore, have a tendency to be less good also, and, on the whole, it is not easy to keep the lines through many generations.

Hermaphrodites, segregating females and hermaphrodites. — This type, which has been described by SHULL (l. c.) and by G. and P. HERTWIG (l. c.), has appeared several times in my material, and I have

regarded the explanation to be that the formula for this type of hermaphrodites is X_fY, which through selfing must give:

$$1\ X_fX_f : 2\ X_fY : 1\ YY$$
$$\text{♀} \qquad \text{♀} \qquad \text{(dies)}$$

but the conditions are evidently more complicated in some cases, as will be seen from the following.

One of the F_2-hermaphrodites, S. 2346—1, which has the full sib of the above named F_2-hermaphrodites S. 2984—5 and S. 2984—15 (the different sowing number is owing to the F_2-material having been sown through two years, 1925 and 1926) was selfed, and segregated females and hermaphrodites, which were intercrossed and crossed with a related male. The result appears from Fig. 13.

It is seen that the two sowings, S. 3821 and S. 3824, show quite different segregations, which are not easily understood, but I suppose that the explanation must be that here again autosomal hermaphrodite-inhibiting genes have accidentally been accumulated in one of the females, being absent in the other. When the three sister plants, S. 2990—14, S. 2990—15 and S. 2990—12, are crossed to the related male, S. 2992—1, they give very different results (see Fig. 13). But also from these segregations it will be obvious that the female 2990—14, which gave only a single hermaphrodite with 2990—8, contains some hermaphrodite-inhibitors, since it segregated only 18 hermaphrodites among 1921 individuals.

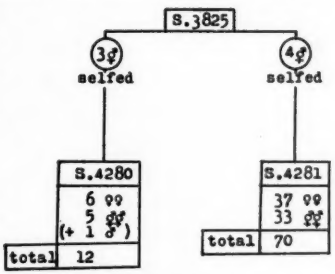


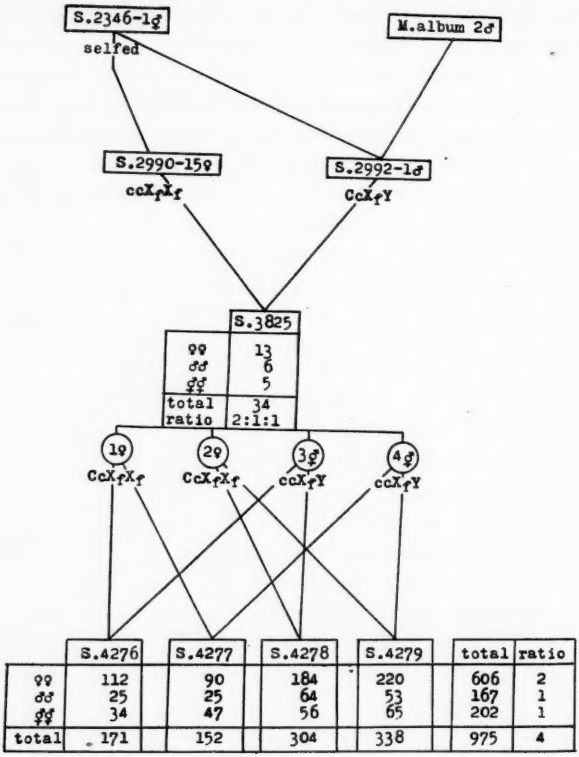
Fig. 14.

In Fig. 13 we find a case, in which, in contradiction to SHULL's results, a hermaphrodite used as mother, transfer the hermaphroditism to the offspring, viz. in the sowing S. 2992.

Two hermaphrodites of S. 3825 were selfed and segregated females and hermaphrodites again, as shown in Fig. 14.

The single male in S. 4280 was unexpected, and it is a question how to explain its appearance. It may be, that SHULL is correct in his view that a mutation has happened, but another possibility cannot be excluded, viz. that the *Y*-chromosome in this male, through crossing-over between *X* and *Y* in the hermaphrodite S. 3825—3, has been altered in strongly male direction. The occurrence, in *Melandrium*, of the gene for »*abnormal*» in both *X* and *Y* supports the view that

crossing-over between *X* and *Y* is possible. Whether a mutation has occurred or a crossing-over the *Y*-chromosome in the unexpected male in S. 4280 must be supposed to have been altered in a more strongly male direction, the strongly female hermaphroditic *X* being hereafter



	S.4276	S.4277	S.4278	S.4279	total	ratio
♀♀	112	90	184	220	606	2
♂♂	25	25	64	53	167	1
⚥⚥	34	47	56	65	202	1
total	171	152	304	338	975	4

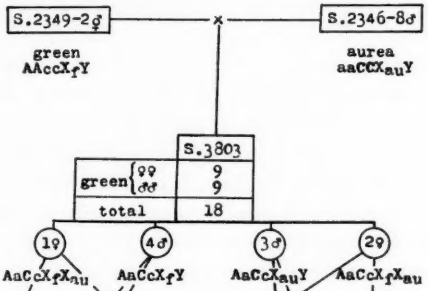
Fig. 15.

unable to pull the development in the *XY*-individual in hermaphroditic direction.

That the *Y*-chromosome of this unexpected male is really strongly male, is supported by further diallel crossings, in which this male is implied (see table 12). Hence I am unable to verify the hypothesis of G. and P. HERTWIG (l. c.) that these single males from hermaphrodites are genetically hermaphrodites.

An autosomal inhibitor of hermaphroditism. — It is possible, indeed, in the inbred material to show a rather simple inheritance

of the hermaphroditism, at least in my material. It is necessary, only, to suppose one autosomal inhibitor of hermaphroditism in large parts of my material. This inhibitor I have called *C*, and as we will see in the following the adoption of this gene is sufficient to explain many segregations, also when the inheritance of hermaphroditism is combined with the inheritance of the *aurea* gene. The effect of *C* is that it pulls the development in male direction, so that CX_fY-individuals are not hermaphrodites but males.



	4247	4250	total	calculated (8:6:1:1)	4248	4249	total	calculated (7:6:1:1)
green ♀♀	311	561	872	846.5	275	613	888	897.8
green ♂♂	204	426	630	634.9	217	575	792	769.6
green ♀♂	28	69	97	105.8	30	75	105	128.3
aurea ♂♂	43	51	94	105.8	35	104	139	128.3
total	586	1107	1693	1693.0	557	1367	1924	1924.0

Fig. 16.

Fig. 15 shows the formulæ of one part of the material, some of which has already been touched upon in the foregoing.

The males and the hermaphrodites in S. 4276—S. 4279 ought to come out in equal number. The surplus of hermaphrodites, 202 against 167 males, is not very far from the expectation. The surplus of females is, as we know, very common in many *Melandrium*-segregations.

The next Figure, 16, shows the inheritance of hermaphroditism in connection with the *aurea*-gene.

On the supposition that the *aurea* male, S. 2346—8, had the inhibitor of hermaphroditism, *C*, present in double dose, and that the hermaphroditic male, to which it was crossed, S. 2349—2, had the *aurea*-inhibitor, *A*, in conformity with Fig. 2, the whole segregation is explained. Through crossing the two individuals the offspring, S. 3803, must be green, and no hermaphrodites can appear, since the formulæ for all females must be $AaCcX_fX_{au}$, while the males are of two kinds, $AaCcX_fY$ and $AaCcX_{au}Y$. It is, however, impossible to observe on the males, which of the two formulæ they possess, as both are green and pure males.

Two males were crossed, diallelic, to two female sibs and gave four families, whose segregations are shown in the pedigree. A male individual of the type $AaCcX_{au}Y$, crossed to a female, $AaCcX_fX_{au}$, must give lethality for $^1/_8$ of the females, $aaX_{au}X_{au}$ being lethal, whether *C* is present or not. The segregation must, therefore, be 7 green females : 6 green males : 1 green hermaphrodite : 1 *aurea* male.

The other male type, however, $AaCcX_fY$, gives no lethal daughters and must give segregations of the type 8 green females : 6 green males : 1 green hermaphrodite : 1 *aurea* male.

Though there is not much difference between the two segregations the agreement is best, when to the one male is attributed the X_{au}-formula and X_f to the other; and it is seen in the pedigree, Fig. 16, that the segregations are in good accordance with the expectations. As the numbers are rather large covering in all 3617 individuals, some significance must be ascribed to the experimental results.

Rather interesting results were obtained from a diallel cross between four different genotypes, the formulæ of which were corroborated through the segregations.

The unexpected male in the offspring S. 4280 (see the pedigree on Fig. 14) was supposed to possess a strongly male *Y*, which was able to give males even in combination with the strongly female (hermaphroditic) *X*, which I have called X_f. The formula of this plant was $AAX_fY_{strongly\ male}$, *A* as we know being the inhibitor of *aurea*. This male, S. 4280—1, was used for crossing (see Table 12).

The second plant used in the diallel was a female of the hermaphroditic line, which segregated females and hermaphrodites, viz. S. 4281—1 (cómpare Fig. 14). As already shown (on page 157) this female type must be homozygous as to X_f. The total formula is AAX_fX_f.

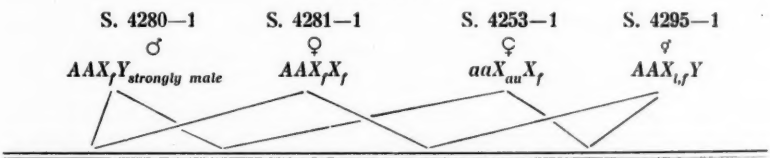
The third plant implied in the diallel was the female, S. 4253—1

(see Fig. 2), which, as we know, had $aaX_{au}X$, since it was a green female from the *aurea*-line. One of the *X*'es, therefore, is an *aurea*-*X*, X_{au}; as to the other the pedigree shows that this *X* has originally come from *Melandrium album* ♂ 2. As in the genealogical tree hermaphrodites have been present in all earlier generations, viz. in S. 2349, S. 2989, S. 3806, as also in S. 4253 itself, it is probable that this *X* is an X_f, the formula hence being $aaX_{au}X_f$.

The fourth individual, S. 4295—1, was a hermaphroditic male from the constant hermaphroditic line (on Fig. 11) with formula $AAX_{l,f}Y$, the *X* containing the hermaphroditic *f*-gene as well as a lethal.

The result of this diallel crossing appears from Table 12.

TABLE 12.



	S. 4771	(theor.)	S. 4769	(theor.)	S. 4770	(theor.)	S. 4768	(theor.)
♀♀	96	(1) AA X_fX_f	400	(1) Aa X_fX_{au} and X_fX_f	732	(1) AA $X_fX_{l,f}$	378	(2) Aa $X_{au}X_{l,f}$ and $X_fX_{l,f}$
♂♂	23	(1) AA $X_fY_{str.\ male}$	226	(1) Aa $X_{au}Y_{str.\ male}$ and $X_fY_{str.\ male}$	2	—	160	(1) Aa $X_{au}Y$
⚥⚥	5	—	5	—	557	(1) AA X_fY	144	(1) Aa X_fY
total	124		631		1291		682	2728

It is seen that there is a good agreement between the formulæ of the four plants and the segregations, and that the four sowings from the diallel crossing give characteristic differences as to the segregations. S. 4771 and S. 4769 give females and males, S. 4770 females and hermaphrodites, and S. 4768 females, males and hermaphrodites in the proportion ab. 2 : 1 : 1.

Also in this material, however, there are a few individuals, 12 out of 2728, which cannot be accounted for.

The number of individuals in Figs. 11—16 and on Table 12 is altogether 10—11000, whose sex has been determined for the purpose of clearing up the inheritance of hermaphroditism. Besides, some thousands other individuals have been used in this investigation. As they show the same features, however, as those already discussed, and as they are of more scattered origin, it is of no special interest to go into their details.

As is apparent from the pedigrees the features concerning the inheritance of hermaphroditism in *Melandrium* are rather clear, the most disputable problem being the origin of the single individuals of exceptional sex. The main lines, however, are unquestionable, the sex being determined by the strength of sex-genes in the different *X*'es and *Y*'s and furthermore by autosomal genes, pulling in male, respectively female direction. In this respect there is full accordance with a so distant organism as the fish *Lebistes* (WINGE 1930 b), where not only hermaphrodites but even *XX*-males could be produced, through the selection of types with weakly female *X*'es and with autosomes pulling in male direction.

VII. OTHER SEX-LINKED GENES.

It is doubtless that, besides the genes already quoted, there exist, in *Melandrium*, several other genes in the sex-chromosomes.

Already in the F_1-generation this was obvious.

F_1 was made by crossing the two pure species in both directions, *album* ♀ with *rubrum* ♂, and *rubrum* ♀ with *album* ♂. When the F_1-plants were classified as to sex and flower colour, and as to their flower size, it was found that the reciprocal crosses gave a different result (see Table 13).

TABLE 13.

	dark red		medium red		pale red		total	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
M. rubr. ♀ × *alb.* ♂ [1] ...	34	141	50	46	133	58	217	245
M. alb. ♀ × *rubr.* ♂ [2] ...	563	262	23	49	83	212	669	523

[1] ♂♂ mainly with larger flowers than ♀♀.
[2] ♂♂ mainly with smaller flowers than ♀♀.

This cannot be cytoplasmatic inheritance, but must be *X*- or *Y*-linked inheritance. But I did not go further with these characters, as they are difficult to work with.

The writer wishes to express his sincere thanks to Dr. J. CLAUSEN for his valuable assistance.

VIII. SUMMARY.

In plants, only two cases were known in which genes have been demonstrated in the sex-chromosomes, the first being the *X*-linked »*angustifolia*»-gene in *Melandrium*, demonstrated by BAUR and SHULL in 1912—1914. This gene, as I have shown later on (1927), was lethal to pollen, so that *angustifolia* males gave only male offspring. Subsequently this *Melandrium* type has been lost.

The second case was described by me in 1927. At that time I believed to have demonstrated a »*chlorina*»-gene in the *Y*-chromosome of *Melandrium*, as *chlorina* plants were always males, and because I did not obtain any *chlorina* females by backcrossing *chlorina* males with their mothers. The present investigations, however, have proved that the gene is *X*-linked and lethal to females possessing the gene in both *X*'es. As *aurea*-types generally possess a lethal gene, killing the homozygous type, I think it better to alter the name of the gene and call it »*aurea*» (*au*).

All *aurea* plants are males. On crossing a heterozygous female $X_{au}X$ with an *aurea* male $X_{au}Y$, the offspring will show a 1 : 1 : 1-segregation, viz. green females, green males and *aurea* males. About 5000 *aurea* males have been counted in 1 : 1 : 1-segregations, but no *aurea* females.

Besides the *aurea*-gene three other genes have been demonstrated in the sex-chromosomes of *Melandrium*, partly in *X* and partly in *Y*:

»*Abnormal*» (*n*), is a recessive gene to be found either in *X* or in *Y*, the allelomorph (*N*) giving normal plants. All possible types have been produced, X_nX_n, X_nY_n, X_nX_N, X_nY_N, X_NY_n, X_NX_N, and X_NY_N. By crossing the different types, the following segregations can be produced: 1) normal males and abnormal females; 2) abnormal males and normal females; 3) all abnormal; 4) all normal. More than 3000 abnormal plants have been sex-determined without revealing any deviation from the theory.

»*Variegated*» is a recessive type. An inhibitor of variegated is demonstrated in the *Y*-chromosome, all true variegated being females. About 1400 variegated plants have been sex-determined.

An *X*-linked gene for »*hermaphroditic males*» has been shown to be present in some pedigrees. But, when the material is outcrossed to unrelated types, the *X*-linked mode of inheritance is disturbed, because the sex to a very high degree is a question of balance between sex-determining genes in all chromosomes.

Constant hermaphrodites, whose existence is explained by balanced lethality, were demonstrated, while another hermaphroditic type segregated females and hermaphrodites.

The sex of *Melandrium* is determined by the strength of sex-genes in the different *X*'es and *Y*'s, and furthermore by autosomal genes, pulling in male or female direction. An autosomal gene, *C*, pulling in male direction, has been demonstrated.

An autosomal inhibitor of »*aurea*» is demonstrated (*A*), as are also three autosomal inhibitors of »*abnormal*» (*G*, *H*, *I*), and three autosomal inhibitors of »*variegated*» (*L*, *M*, *O*). Through appropriate selection these inhibitors can be discarded, whereby simple segregations will result.

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NOTE ON THE CYTOLOGY OF SOME APOMICTIC POTENTILLA-SPECIES

BY *ARNE MÜNTZING*
HILLESHÖG, LANDSKRONA, SWEDEN

INTRODUCTION.

IN a previous paper (MÜNTZING 1928) I have reported the results of experimental investigations in the *Potentilla*-groups *Argenteæ*, *Collinæ* and *Aureæ vernales*. Simultaneously a preliminary orientation as to the cytological conditions was given. Though these investigations — on account of other work — have but little advanced the present paper communicates some further cytological observations. Though still fragmentary they have an actual interest on account of the cytological results obtained by TISCHLER (1928, 1929 a, 1929 b) and SHIMOTOMAI (1930), working at the same genus.

The cytological results obtained by me in 1928 were in the main the following: Different biotypes of *Potentilla argentea* proved to differ in chromosome number, one or probably two being diploid with the basic number 7, another biotype having a considerably higher number which could not exactly be determined. One *Crantzii*-biotype was hexaploid, having $2n = 42$. *Tabernæmontani*- and *collina*-biotypes, too, had high chromosome numbers ($2n =$ abt. 40). Further, in some *Crantzii*-biotypes irregularities during the reduction division were observed.

The present cytological results comprise determinations of the somatic chromosome numbers (root tips) of 19 biotypes and observations of the meiosis of the pollen mother cells in diploid and hexaploid *argentea* and duodecaploid *Tabernæmontani*.

The root tips and buds were fixed in NAWASCHIN's fixative, the buds being at first treated with CARNOY for some minutes. The sections were cut 14—20 μ thick and stained with NEWTON's gentian violet. For *Potentilla* this stain is obviously superior to haematoxylin.

SOMATIC CHROMOSOMES.

No differences in the size of the chromosomes were observed. However, better fixatives will probably reveal morphological differen-

tiations. As is seen in fig. 2 the attachment constrictions are in some chromosomes probably median, in others subterminal. Occasionally satellites were visible.

The following chromosome numbers were determined (table 1).

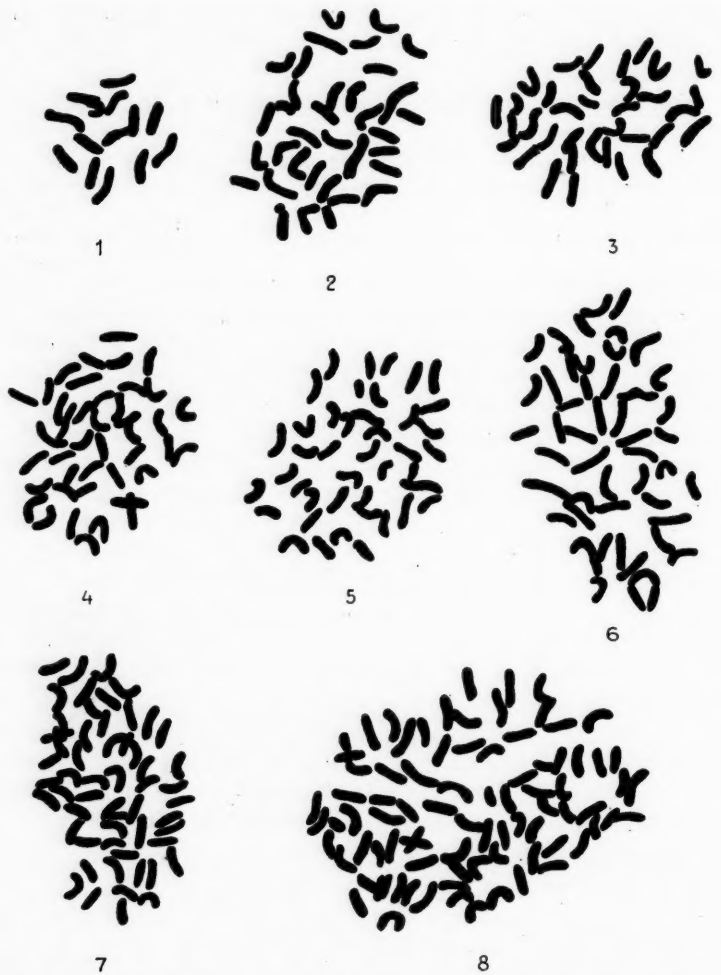
TABLE 1. *Somatic chromosome numbers.*

Species and biotype		Origin	Biotype symbols	Somatic chromosome number
Tabernæmontani	A	The province of Västergötland, Sweden	T—A [1]	42
»	B	The isle of Öland, Sweden	T—B	± 84
argentea	A	Dalby, Sweden	A—A	14
»	B	Lomma, »	A—B	42
»	C	The Bot. Garden, Basle	A—C	14
»	D	» » Lyon	A—D	42
»	E	» » Iasi	A—E	56
»	F	» » Brno	A—F	42
»	G	» » Edinburgh	A—G	14
collina	A	The isle of Gotland, Sweden	C—A	42
»	B	The Bot. Garden, Lyon	C—B	35
»	C	The isle of Öland, Sweden	C—C	42
»	D	» » »	C—D	42
»	E	» » »	C—E	42
»	F	» » »	C—F	42
»	G	The Bot. Garden, Uppsala	C—G	35
Crantzii	A	The province of Västergötland, Sweden	Cr—A	42
»	B	The Bot. Garden, Helsingfors	Cr—B	42
»	C	» » Nantes	Cr—C	49

The somatic chromosome numbers vary between 14 and 84, all being multiples of seven (figs. 1—8). This is in accordance with the previous cytological investigations of the genus. Two other facts, however, deserve special consideration: 1) The occurrence of odd multiples ($2n = 35$ and 49) in two *collina*- and one *Crantzii*-biotype. 2) The occurrence of widely different chromosome numbers in the same species.

The experimental investigations (MÜNTZING 1928) have proved apomictic propagation (pseudogamy) for different biotypes of *P. argen-*

[1] The biotypes T—A, T—B and Cr—A were kindly put at my disposal by Dr. G. TURESSON, Landskrona.



Figs. 1—8. Somatic plates (× 3530). Fig. 1, diploid *argentea* (A—G); fig. 2, pentaploid *collina* (C—G); fig. 3, pentaploid *collina* (C—B); fig. 4, hexaploid *collina* (C—E); fig. 5, hexaploid *Tabernæmontani* (T—A); fig. 6, heptaploid *Crantzii* (Cr—C); fig. 7, octoploid *argentea* (A—E); fig. 8, duodecaploid *Tabernæmontani* (T—B).

tea, *collina* and *Tabernæmontani*. Pollination is necessary for seed development, but all progenies are purely maternal in respect of morphological characters as well as chromosome number. Only very few indi-

viduals in progenies after selfing or crossing were divergent, indicating that the apomixis was not absolute. However, when this was written these few exceptional plants were not full-grown. Later on they proved to be either modifications or obvious intermixtures. Only in progenies of one diploid *argentea* (A—C) it remains to be settled wether observed morphological differences are only modificatory or due to genotypical causes.

Parallel to the apomictic propagation the biotypes investigated were characterized by strict morphological constancy in the seed progenies and further by partial pollen sterility (with the exception of two diploid *argentea*-types). On account of this and with reference to the investigations of other apomictic genera the conclusion was drawn, that the *Potentilla*-types in question offered a new example of heterozygosity fixed by apomixis.

The finding of pentaploid *collina*- and heptaploid *Crantzii*-biotypes supports this conclusion very much. It is most probable that these biotypes are the result of crosses between parents with different chromosome number and also qualitatively different genetic constitution. This is of particular interest as, from a taxonomical point of view, *collina* is supposed to have a hybrid origin (cf. MÜNTZING 1928, p. 267). However, most *collina*-biotypes investigated are hexaploid. SHIMOTOMAI (1930) gives the same number for *collina* and indeed for all types belonging to the groups *Collinæ*, *Argenteæ* and *Aureæ* investigated by him. Further he points out the fact that those *Potentilla*-groups which are regarded as old and primitive and which are said to have existed already in the tertiary period (e. g. the groups *Fruticosæ* and *Rupestres*) are characterized by low chromosome numbers ($2n = 14$) and a relatively limited and decreasing geographical distribution. In contrast to this, the big group *Conostylæ* has numerous representatives and comprises almost two thirds of the species of the genus. These species are »jung und polymorph, und offenbar jetzt in ihrer vollen Entwicklung begriffen» (SHIMOTOMAI 1930, p. 7). It is remarkable that among these species the chromosome numbers are generally high, but the following statement by SHIMOTOMAI (1930, p. 7) is not quite correct: »Es gibt in der Tat keine Art, bei der die Grundzahl auftritt; alle Arten weisen mehr oder weniger hochgesteigerte polyploide Chromosomenzahlen auf». In my previous paper on *Potentilla* (1928) I reported the occurrence of two diploid *argentea*-races, and in 1929 (a) TISCHLER describes a diploid *opaca*. During the present investigation I found a third diploid *argentea*. — These diploid biotypes are especially in-

teresting after the investigations of SHIMOTOMAI, as according to his arguments types with low chromosome number are to be regarded as old and primitive. — The question may be further elucidated by investigations of the chromosome numbers in other *argentea*-races from different localities.

It is noteworthy that hitherto only diploid, hexaploid and octoploid *argentea*-types have been found but no tetraploid ones. Anyhow, *P. argentea* L. offers a good instance of the occurrence of different chromosome-races within the same species. *P. argentea* is relatively polymorphic and on account of the mode of reproduction a great multitude of different constant types might be distinguished. Between the chromosome numbers and the morphological characters there seems to be a correlation so far, that highly polyploid types are more vigorous than types with low chromosome numbers. The contrast was especially marked between a prostrate and tiny diploid biotype (A—A), collected at Dalby, province of Scania, south Sweden, and a high and erect hexaploid type, growing only about 20 km. from the other locality (at Lomma). The octoploid *argentea* (obtained from the Bot. Garden of Iasi) was still more vigorous.

Those habitually rather different biotypes must, however, all be regarded rather typical *argentea*, and there seems to be no reason to split *P. argentea* into a great number of »species» of the *Hieracium*-type (cf. MÜNTZING, TEDIN and TURESSON 1931), though this might certainly be easily done.

Also in the other species investigated different chromosomal strains were found, in *collina* five hexaploid and two pentaploid ones, in *Crantzii* two hexaploid and one heptaploid and in *Tabernæmontani*, finally, one hexaploid and one duodecaploid. This latter type was very big and coarse and seems to show that, as in *argentea*, vigour is proportional to chromosome number.

Also TISCHLER (1929 a) found intraspecific chromosomal variation. In *Potentilla opaca* one diploid and one tetraploid race were detected. Further, for *P. multifida* TISCHLER gives the haploid number 7, SHIMOTOMAI counted $2n = 42$. *P. villosa* ZIMM. is probably synonymous with *P. Crantzii* BECK. According to TISCHLER *P. villosa* has $n = 7$; my *Crantzii*-strains were hexaploid and heptaploid.

Though in certain cases the taxonomical classification of the types investigated may be difficult, it is evident that the *Potentilla*-groups in question are characterized by polymorphism also in regard to the chro-

mosome multiples, and that quite different numbers may occur in the same species.

Apomictic plants are on an average characterized by high chromosome numbers (cf. ROSENBERG 1930, p. 59), but inversely, polyploid types in a plant genus must not necessarily be apomictic. However, especially in such cases where different strains within the same species have different chromosome numbers (see the list of CLAUSEN 1931, p. 68) it ought to be investigated wether all of these strains are really sexual. Intraspecific chromosome multiples suggest autopolyploidy, and this will probably be correlated with multivalent formation and partial sterility. Partially sterile types which are able to survive in nature may be suspected of apomictic propagation. Wether this is the case may be settled by castration experiments. But if the seed development is pseudogamous also pollinations with foreign pollen and observations of the progeny are necessary. Such experiments have been carried out only in a few cases.

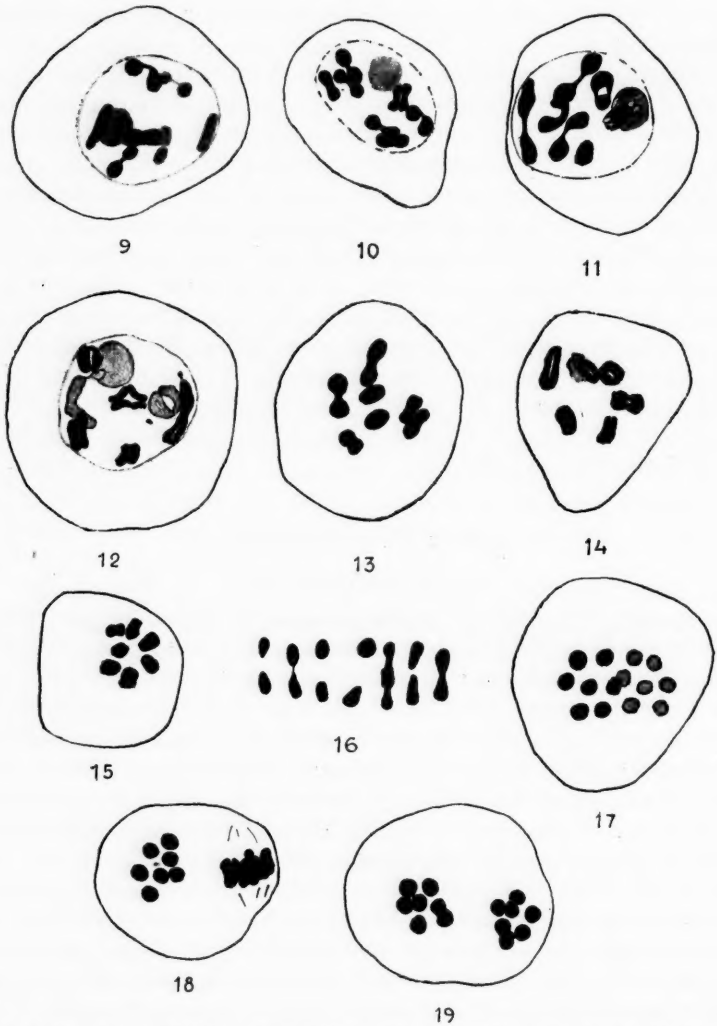
MEIOTIC DIVISIONS.

a) DIPLOID ARGENTEA.

In one of the diploid *argentea*-strains (A—A) some good slides were obtained, showing different meiotic stages of the pollen mother cells. The divisions seem to be completely normal (figs. 1—18). At diakinesis the gemini are usually associated by one or sometimes by two terminal chiasmata. Probably there is a reduction in number of chiasmata from diplotene to diakinesis (cf. NEWTON and DARLINGTON 1929, LAWRENCE 1929). Consequently the stage with two chiasmata is prior to that stage when the paired chromosomes have swung apart at one end and are associated by only one terminal chiasma. In the centre of fig. 12 this kind of association is evident, all four chromatids being clearly visible. During metakinesis (figs. 13—14) the chromosomes contract and get the typical shape of first metaphase bivalents (figs. 15—16). The hetero- and homotypic divisions are normal (figs. 17—18).

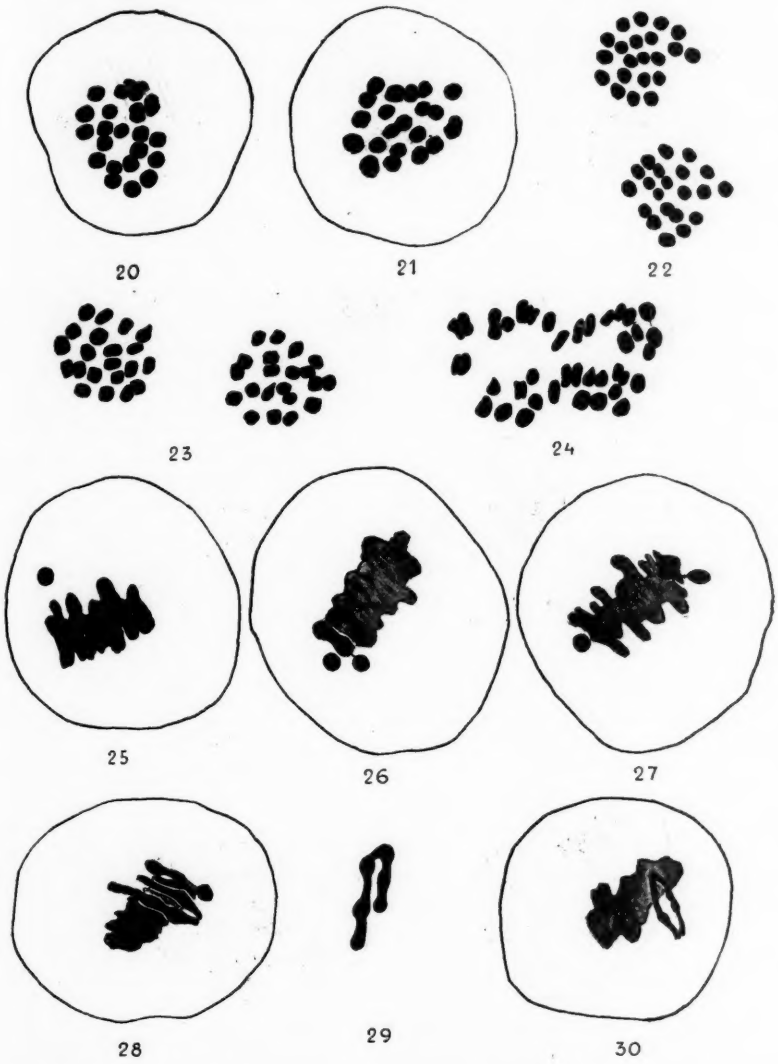
b) HEXAPLOID ARGENTEA.

From one hexaploid *argentea* (A—B) slides were obtained, showing the hetero- and homotypic divisions. The divisions give the impression of being chiefly normal. In several cases heterotypic anaphases were observed, showing the normal distribution 21—21 (figs. 22—23). Polar



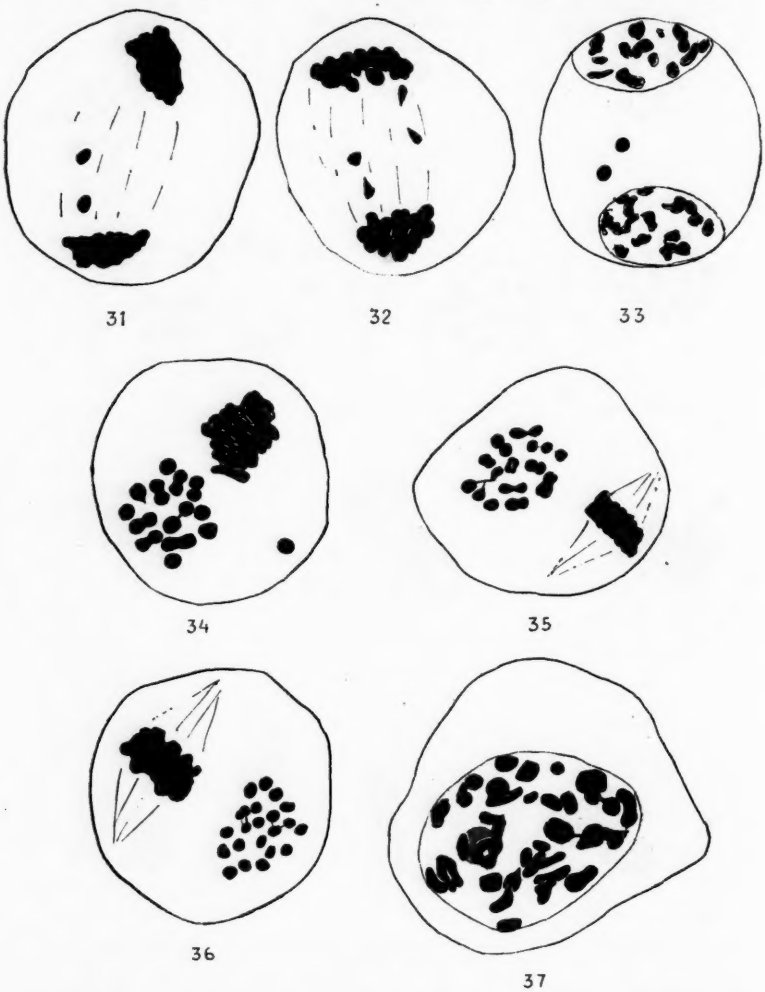
Figs. 9—19. Meiotic divisions in diploid *argentea* (× 3530). Figs. 9—12, diakinesis; figs. 13—14, metakinesis; fig. 15, heterotypic metaphase, polar view; fig. 16, heterotypic metaphase, side view (separately drawn); fig. 17, heterotypic anaphase; figs. 18—19, homotypic metaphase.

views of the first metaphase, however, indicate that sometimes multiple association takes place. In fig. 20, above, there is a possible



Figs. 20—30. Meiotic divisions in hexaploid *argentea* (× 3530). Figs. 20—21, heterotypic metaphase, polar view; figs. 22—23, heterotypic anaphases; fig. 24, commencing heterotypic anaphase (in part separately drawn); figs. 25—28 and 30, heterotypic metaphase, side view; fig. 29, chromosome chain from heterotypic metaphase.

quadrivalent and in fig. 21 possibly one hexivalent and three or four quadrivalents, but this association might be casual and due to bad fixation. In fig. 24, however, a commencing anaphase was analyzed,



Figs. 31—36. Meiotic divisions in hexaploid *argentea* (continued); fig. 37, diakinesis in duodecaploid *Tabernæmontani* (× 3530). — Fig. 31, heterotypic anaphase with lagging chromosomes; fig. 32, heterotypic anaphase, division of lagging univalents; fig. 33, interphase, two chromosomes eliminated; figs. 34—36, homotypic metaphase.

which gives a somewhat stronger evidence of a previous multiple association, though the distribution of the chromosomes even in this case seems to be the normal 21—21. E. g. to the extreme left in the anaphase groups the separating chromosomes of one bivalent are intimately connected with those of another one. Evidently these two bivalents have formed a quadrivalent of the same type as those observed in *Prunus* (DARLINGTON 1928, p. 232, text-fig. 18: 23—33) and in *Empetrum hermaphroditum* (HAGERUP 1927, p. 8, fig. 3 d). In the same anaphase the third chromosome on the left is most probably a bivalent. Farthest to the right there seems to be another bivalent if the connection is not an artefact. — Fig. 25 shows a univalent being detached from the rest of the metaphase chromosomes. In figs. 26—30 there are clear cases of multiple association at the heterotypic metaphases. Figs. 26 and 27 show the simultaneous presence of one univalent and one trivalent. In fig. 29 the chain consists of at least 4 chromosomes, in fig. 30 there is a probably quadrivalent ring.

Such multiple association may lead to lagging and elimination of one or more chromosomes, as is evident from figs. 31—34. Sometimes the lagging univalents seem to divide (fig. 32).

Homotypic divisions were observed showing a more or less pronounced »secondary pairing». In fig. 34 there is a homotypic metaphase plate where the 20 chromosomes (one chromosome eliminated at the heterotypic division) form 6—7 double chromosomes and 8 or 6 single ones. In fig. 35 there seems to be association in $2_{III} + 6_{II} + 3_{I}$, and in fig. 36 there are possibly one trivalent and one bivalent association.

In cases where multiple association occurs at the heterotypic division, resulting in the passing of bivalents or multivalents to the same pole, »secondary pairing» is to be expected also at the homotypic division, provided that the homologous chromosomes do not separate during interphase. Such »secondary pairing» has been observed in the tetraploid *Empetrum* (HAGERUP 1927), in *Prunus* (DARLINGTON 1928, MEURMAN 1929), in *Dahlia* (LAWRENCE 1929) and in *Pyrus* (DARLINGTON and MOFFETT 1930).

In the present case, however, the true nature of the association at the homotypic metaphase is uncertain, as in the *diploid argentea* in one case a similar association was observed at the homotypic metaphase (fig. 19). The association in this case was evidently due to bad fixation and suggests that the associations in the hexaploid (especially such as in fig. 34) may be artefacts and not caused by chromosome homo-

logy. — But however this may be, the clear multiple associations at the heterotypic division cannot be artificial. Then, in some cases at least real secondary pairing should probably occur also at the homotypic metaphase. — It is noteworthy that at the first metaphase no higher multiples than quadrivalents or hexivalents seem to be formed, and at the homotypic metaphase association takes place between two or three chromosomes, not more. This is in accordance with the hexaploidy, if the six sets of seven are more or less homologous.

Also TISCHLER (1928) has evidently observed association of chromosomes in polyploid *Potentilla*-species. He writes (l. c. p. 330): »Die Chromosomen neigen auch bei sonst gut fixierten Präparaten hier derart zur Verklumpung, dass selbst in den gut zählbaren Metaphasen der heterotypen Teilung meist bei einigen die Grenzen gegenüber den Nachbarchromosomen schlecht gewahrt waren».

c) DIAKINESIS IN THE DUODECAPLOID TABERNAEMONTANI.

A few pollen mother cells in diakinesis were seen in the *Tabernæmontani*-line T—B, which had $2n = \pm 84$ (fig. 8). Though the fixation was not very good multiple association was obvious (fig. 37). Within the nucleus (which was complete) 31 separate bodies of a very varying size could be discerned. Probably these bodies represent univalents, bivalents, trivalents and still higher multiples. Near the nucleolus there is a chain consisting of at least 4 chromosomes.

It is obvious that the partial pollen sterility in the polyploid *Potentilla*-strains (cf. MÜNTZING 1928, p. 269 and the present paper, table 1) is at least in part due to chromosome irregularities leading to the formation of non-viable gamete combinations. On the other hand, the diploid types have a normal reduction division and complete pollen fertility. The pentaploid *collina*-strains C—B and C—G show the highest degree of sterility. C—B is completely male-sterile. This sterility, however, is diplontic (MÜNTZING 1930, p. 314—322), the whole anthers being contabescent. In the other pentaploid, C—G, it ought to be possible to study meiosis in the pollen mother cells. Probably such a study will reveal hybrid chromosome garnitures. — According to TISCHLER (1929 a) a supposed hybrid, *Potentilla verna* L. × *opaca* L., showed $7_{II} + 14_{I}$ at diakinesis.

The occasional multiple association of the chromosomes in hexaploid *argentea* suggests that the different sets of seven chromosomes are

to a certain degree homologous. Further, the fact that widely different multiples exist in the same agamospecies indicates autopolyploidy at least in some cases. However, e. g. in the *collina*-group, where the morphological variation is very great, it is probable that species crossing and allopolyploidy have played an important rôle. These problems can only be solved by experimental work, involving sexual or partially sexual strains. Such races have not hitherto been found but will be searched for during further investigations.

The present investigation was carried out during a stay at The John Innes Horticultural Institution, London. To the chief and staff of this institution I wish to express my sincere gratitude for help and advice.

SUMMARY.

1) The somatic chromosome numbers in different biotypes of *Potentilla argentea, collina, Tabernæmontani* and *Crantzii* were determined and found to vary between 14 and 84, all being multiples of 7.

2) Previous experimental facts indicated that the apomictic *Potentilla*-biotypes are generally heterozygous. The finding of two pentaploid *collina*-strains and one heptaploid *Crantzii* strongly supports this view.

3) In all the species investigated there is intraspecific variation in chromosome number.

4) Meiotic divisions in the pollen mother cells were observed in diploid and hexaploid *argentea* and in duodecaploid *Tabernæmontani*. In the diploid biotype meiosis is perfectly regular, in the polyploids there is sometimes multiple association of chromosomes. This association, which indicates autopolyploidy, sometimes leads to elimination of chromosomes and hence to partial pollen sterility.

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STUDIES ON THE CHROMOSOME NUMBERS OF THE UMBELLIFERÆ

BY *J. H. WANSCHER*
GENETICS LABORATORY OF THE ROYAL VETERINARY AND AGRICULTURAL COLLEGE, COPENHAGEN

(A preliminary report)

THE large family of the Umbelliferæ has not until the latest years been cytologically investigated, and many years may, perhaps, pass away before the several genera and species are thoroughly studied and compared cytologically. The following article is only a contribution to this scope.

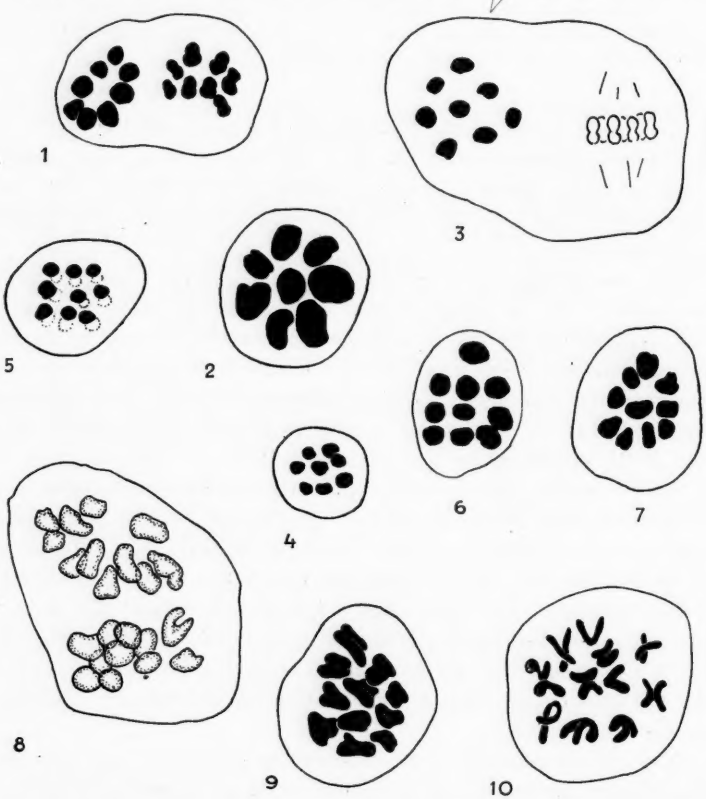
For the purpose of counting the chromosomes I have chosen to investigate meiosis in pollen mother cells. I succeeded in stating with great accuracy the chromosome numbers of twentytwo species, of which nineteen numbers were new. I have been able to check three of the formerly stated numbers: *Anthriscus silvester* HOFFM. (H. E. PETERSEN 1914, WINGE 1917), *Anthriscus cerefolium* HOFFM. and *Myrrhis odorata* SCOP. (both published by EM. MARCHAL 1920).

NAWASCHIN's mixture was employed for fixing, and the young flower buds were immersed in this fixative for abt. 24 hours after a treatment with CARNOY's mixture for about two minutes. Sections were cut 10 μ thick and stained with gentian violet, which proved useful for this material. In a few cases (*Apium* and others) the stain was extracted rather fast again during the process of destaining, but otherwise this proved to be an advantage, as the chromosomes of *Apium* were left transparent enough for counting the heterotypic anaphase in side view (see fig. 8).

The chromosomes as they appeared in the heterotypic and homotypic division proved to be of rather different shape and size in the different genera and species. They seemed also to be of at least two well distinguished types, a long and a short type.

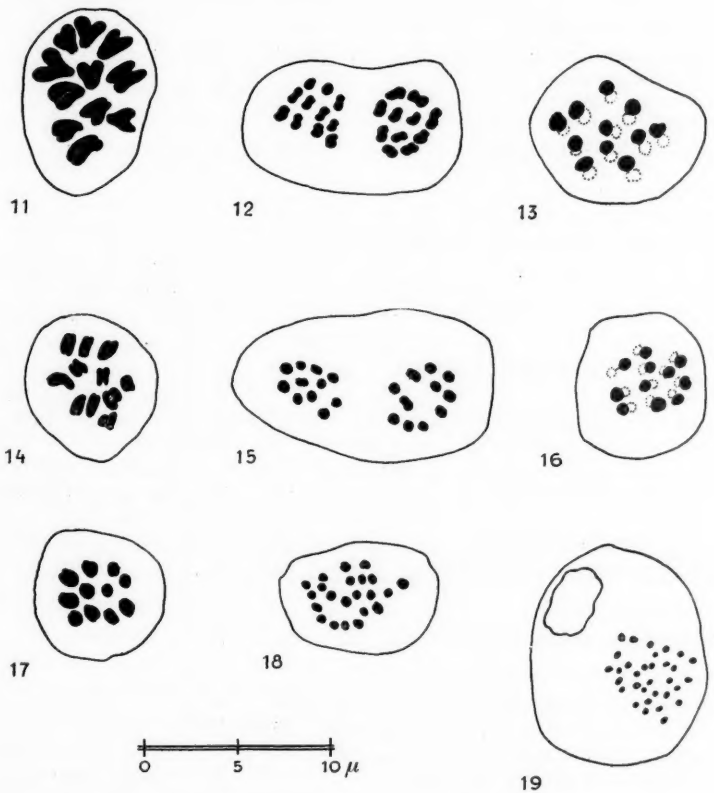
The chromosomes of nineteen species are illustrated in the figures 1—19, all of them are from camera drawings of the meiosis in pollen mother cells ($\times$ 2800). The chromosomes in figs. 8 and 19 are of the short or round type but highly different in size. The figs. 9, 10, 11 and 14 illustrate the chromosomes of the long type as they appear in the

heterotypic metaphase. Some of these nuclear plates clearly demonstrate the bivalency of the chromosomes in the heterotypic metaphase. In a single case, viz. *Athamanta Matthioli*, I found ten bivalent and two univalent chromosomes in a heterotypic nuclear plate of the same type as in fig. 9. A further study of the characters of the chromosomes may, perhaps, be able to inform us whether the size or shape of the chromo-



Figs. 1—19. Camera drawings from the meiosis in pollen mother cells of different species of the Umbelliferæ (× 2800). — *1*, *Anthriscus fumarioides* SPRENG., homotypic metaphase (8 + 8 chromosomes); *2*, *Bupleurum longifolium* L., heterotypic metaphase, (n = 8); *3*, *Sanicula europæa* L., homotypic metaphase, (n = 8); *4*, *Scandix pecten Veneris* L., heterotypic metaphase, (n = 8); *5*, *Anthriscus vulgaris* (PERS.) BERNH., heterotypic metaphase, (9_{II}); *6*, *Carum carvi* L., heterotypic metaphase (n = 10); *7*, *Aethusa cynapium* L., heterotypic metaphase, (n = 10); *8*, *Apium graveolens* L., heterotypic anaphase in side view, (11 + 11 chromosomes); *9*, *Archangelica litoralis* (FR.) AGARDH, heterotypic metaphase, (11_{II}); *10*, *Athamanta Matthioli*

somes are of any taxonomic value. Within the same genus the chromosomes are probably of the same type, as are the chromosomes in four species of *Anthriscus* and two species of *Chærophyllum* which I have studied. *Anthriscus* has chromosomes of the short type, and *Chærophyllum* has chromosomes of the long type. (See figs. 1, 5 and 11.)



WULF, early heterotypic anaphase, (11_{II}); *11*, *Chærophyllum hirsutum* L., heterotypic metaphase, (11_{II}); *12*, *Conium maculatum* L., homotypic metaphase, (11 + 11 chromosomes); *13*, *Peucedanum ostruthium* KOCH (= *Imperatorium ostruthium* L.), (11_{II}); *14*, *Meum athamanticum* JACQ., heterotypic metaphase, (11_{II}); *15*; *Oenanthe pimpinelloides* L., homotypic metaphase, (11 + 11 chromosomes); *16*, *Sium lancifolium* BIEB., heterotypic metaphase (11_{II}); *17*, *Trochiscanthes nodiflorus* KOCH, heterotypic metaphase, (n = 11); *18*, *Molopospermum cicutarium* DC., heterotypic metaphase, (n = 22); *19*, *Hydrocotyle vulgaris* L., homotypic metaphase, (n = ca. 32).

TABLE 1. *List of chromosome numbers of the Umbelliferæ. The numbers without author are new. Genera arranged mainly according to* ENGLER und GILG (*1924*).

Group and name	Figure no.	n =	Series	Author
Hydrocotyle group				
Hydrocotyle vulgaris L.	19	32 or 33	A?	
Sanicula europæa L.	3	8	A	
Caucalis group				
Torilis anthriscus GMEL.		8	A	OGAWA 1929
Scandix group				
Scandix pecten Veneris L.	4	8	A	
Anthriscus fumarioides SPRENG.	1	8	A	
» *silvester* HOFFM.		8	A	H. E. PETERSEN 1914 WINGE 1917 the present author 1931
» *vulgaris* (PERS.) BERNH.	5	9	A	
» *cerefolium* HOFFM.		9	A	EM. MARCHAL 1920 the present author 1931
Myrrhis odorata SCOP.		11	B	EM. MARCHAL 1920 the present author 1931
Chærophyllum hirsutum L.	11	11	B	
Molopospermum cicutarium DC.	18	22	B	
Smyrnium group				
Conium maculatum L.	12	11	B	
Carum group				
Bupleurum longifolium L.	2	8	A	
Carum carvi L.	6	10	B?	
Apium graveolens L.	8	11	B	
Petroselinum sativum HOFFM.		11	B	OGAWA 1929
Cicuta virosa L.		11	B	OGAWA 1929
Sium lancifolium BIEB.	16	11	B	
Aegopodium podagraria L.		abt. 20	B?	WINGE 1917
Seseli group				
Aethusa cynapium L.	7	10	B?	
Oenanthe pimpinelloides L.	15	11	B	
Ligusticum acutilobum SIEB. & ZUCC.		11	B	OGAWA 1929

Group and name	Figure no.	n =	Series	Author
Foeniculum vulgare GAERTN. ...		11	B	OGAWA 1929
Meum athamanticum JACQ.	14	11	B	
Athamanta Matthioli WULF	10	11	B	
Trochiscanthes nodiflorus KOCH	17	11	B	
Angelica group				
Archangelica litoralis (FR.) AG.	9	11	B	
Angelica pubescens MAXIM.....		11	B	OGAWA 1929
» *silvestris* L.		11	B	OGAWA 1929
» *sp.*		33	B	OGAWA 1929
Phellopterus litorialis FR. SCHM.		11	B	OGAWA 1929
Ferula group				
Peucedanum japonicum THUNB.		11	B	OGAWA 1929
» *decursivum* MAXIM.		11	B	OGAWA 1929
» *ostruthium* KOCH(= *Imperatoria ostr.* L.)	13	11	B	
Heracleum group				
Pastinaca sativa L.		11[1]	B	OGAWA 1929

[1] FLOID E. BEGHTEL reports this number to be probably eight. He investigated megaspore mother cells. But he does not bring any drawing from nuclear plates and remarks that the counting of the chromosomes is very difficult and uncertain.

In Table 1 all the chromosome numbers are enlisted. The genera are arranged mainly according to ENGLER und GILG (1924). Numbers without author are new.

The numbers obtained were the following: 8, 9, 10, 11, 22, and 32 or 33. As will be seen from the table the numbers may be arranged into two series, an *eight* series (in the following referred to as the A-series) and an *eleven* series (referred to as the B-series). KIN-YA OGAWA (1929) draws the same conclusion from the numbers published by him.

In the A-series I include the numbers 8 and 9 because these two numbers in other cases very often accompany each others, and, as appears from the table, both occur within the genus *Anthriscus*. This series comprises the genera *Sanicula*, *Scandix*, *Anthriscus* and *Bupleurum*, all with diploid species, and perhaps *Hydrocotyle* also, if the chromosome number be 32 ($4 \times 8 = 32$).

To the B-series I refer the numbers 11, 22, 33 and perhaps also 10.

All the remaining genera studied are placed in this series, *Carum* and *Aethusa* (n = 10) with reservation, however.

The taxonomic system of the Umbelliferæ according to ENGLER und GILG (1924) is based on the characters of the fruit, and places the genera in a succession which does not fully agree with the two series A and B. The *Scandix* group contains genera belonging to both series: *Scandix* and *Anthriscus* of series A, *Myrrhis*, *Chærophyllum* and *Molopospermum* of series B. The *Carum* or *Apium* group contains the genus *Bupleurum* of the A-series (n = 8), while all the remaining genera belong to the B-series with the chromosome numbers 10 and 11. In all the remaining groups, as it appears from the table, representatives for only the B-series are found.

A coming investigation of the chromosome numbers together with taxonomic studies on the species and genera may, perhaps, be able to explain the irregularity of the *Scandix* and *Carum* groups and perhaps render a well proved basis for a new taxonomic system and for a replacement of some of the groups and genera and, perhaps, species too.

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TWO LINKAGE GROUPS IN THE GARDEN BEAN

BY *KLAS TJEBBES*
THE HILLESHÖG INSTITUTE FOR SUGARBEET RESEARCH, LANDSKRONA

ACCORDING to several cytologists (KARPECHENKO 1925, WEINSTEIN 1926) the haploid chromosome number in *Phaseolus vulgaris* L. is 11. The number of known genetic factors in this species surpasses by far this figure so that certainly there must exist many linkages between them. The number of analyzed cases of linkage in beans is, however, not very great. Some facts relative to five factors that belong to two linkage groups may be published in the following paragraphs.

I. THE B—R—S LINKAGE.

In earlier publications the present author, in collaboration with H. N. KOOIMAN, (TJEBBES & KOOIMAN I—IX, 1919—1923) has given gene analyses of some Dutch bean varieties. KOOIMAN (1922), in his dissertation, has completed and arranged a part of the material and later on, in a short monograph (KOOIMAN 1926), summarized the factors described by several other geneticists and by us.

The seed coat factors that will be spoken about in this paper are:

A, a basal factor for colour: *aa*-plants have white or green seeds. *A* is a complete dominant, but in some rare cases the colours in the heterozygotes are somewhat paler. *A* itself has no visible effect without other factors cooperating.

B, a chromogen factor causing, in the presence of *A*, a pale yellow pigment, self colour when homozygous, but mottled in the heterozygotes. It does not colour the umbilical ring.

C is also a chromogen factor, causing a greyish yellow when accompanied by *A* only. It is a pure dominant: the heterozygotes are not mottled. It intensifies the yellow caused by *B* to a vivid orange-brown, known from many »brown beans» cultivated on an agricultural scale for their dry seeds. *C*-beans have a brown umbilical ring.

D gives, with *A* only, a greyish white, in some cases a dirty buff. The pigment is more concentrated at the umbilical side of the seed. The ring is also coloured. Together with *B* or *C* or both it makes the colours caused by those factors slightly darker and more greyish.

The pale yellow caused by *B* disappears almost totally when the seeds are cooked. But if *C* or *D* are present the brown pigment remains undissolved.

KOOIMAN (1922, 1926) has described several intensifying factors, *E*, *F* etc., that have a darkening influence upon the colours caused by *B* and *C*, giving rise to dark brown, chocolate and black pigmentations. Those factors do not occur in the material for this article, nor does the factor for constant mottling *M*, and others.

In several of our papers (TJEBBES & KOOIMAN I, 1919; IV, V, 1921; VI, VII, 1922; IX, 1923) the factors *R* and *S* are spoken about. *R* gives with *A* a pale wine-red pigment, that is intensified by *C* to burgundy red (*lie de vin*) and epistatic over the yellow and brown pigments. *S* has no influence on the colours of the yellow and brown type but restricts the extension of the red colour to a system of longitudinal stripes and patches. Both factors are dominants and nearly related. In many varieties *R* and *S* occur simultaneously. If such varieties are crossed with others usually no self-coloured red forms are segregated. In the beginning of our experiments on beans we believed that they were only one factor, which we called *S*. Later on I found some American varieties with uniformly coloured wine-red seeds, where *R* apparently acts separately.

Black seeded beans may possess *R*, accompanied by a factor *Bl*, that changes the red pigment into blue, and eventually by intensifying factors *Z*, *Z'* etc., comparable with the *E*, *F* etc. factors in the yellow and brown series. The pigments caused by *R* and its modifiers are soluble in hot water and disappear when the beans are cooked; if *C* is present the brown colour remains.

Many experiments have shown (KOOIMAN 1922) that the factors *A*, *B*, *C*, *D* and their modifiers segregate and combine freely with each other, but this does not apply to *R* and *S*. In two earlier papers (TJEBBES & KOOIMAN I, 1919; IV, 1921) mention was made of a cross between an orange-brown self-coloured and a red striped variety, showing that one of the colour factors of the yellow series was strongly linked to the factors (or factor: at that time we still believed that *R* and *S* were one) that cause the red striped pattern.

The details of this case of »repulsion» were shortly as follows: the F_1 of the cross had a mottled orange-brown seed coat that at the same time showed red striping. The stripes were broken up somewhat in consequence of the mottling. The second generation of this cross consisted of only three classes: $^1/_4$ self-coloured orange-brown as the one

parent (remaining constant in further generations), $^1/_4$ red striped as the other parent (also constant) and $^1/_2$ of the F_1-type, segregating in the same way in further generations. Apparently this is a case of very close linkage between 3 factors, *R*, *S* and the brown colour factor. That this brown factor is *B* appears from the mottled pattern on the F_1-seeds. The cross may be written: *AABBCCrrss* × *AAbbCCRRSS*. The F_1, *AABbCCRrSs*, forms, out of the possible 8 kinds of gametes, practically only *Brs* and *bRS*, and the F_2 consists of:

$^1/_4$ *AABBCCrrss*, self-coloured orange-brown and constant,

$^1/_4$ *AAbbCCRRSS*, red striped and constant,

$^1/_2$ *AABbCCRrSs*, mottled orange-brown and red striped, segregating.

The *R*—*S* linkage must be very strong. In a great number of crosses of this kind (together about 2500 plants) only twice a self-coloured red occurred. The *BRs*- and *bRs*-gametes seem to be very rare. Perhaps the percentage of crossing-over between *B* and the *RS*-complex was somewhat higher, but in the presence of *C* the classes *BB* and *bb* are difficult to distinguish. *Bb*- (mottled) individuals without *RS* were not found among this material. I draw the conclusion that also the linkage between *B* and *RS* is a very close one.

In order to test this *B*—*R*—*S* linkage another cross was made in which one of the partners was a variety with self-coloured dark burgundy red seeds (Canadian Wonder). It possessed *B*, notwithstanding the presence of *R*, but it lacked *S*. The other partner was a white seeded form from a series described in an earlier publication (TJEBBES & KOOIMAN V, 1921) under No. 13. It was known to possess *R* and *S* cryptomerically [1], but to lack the basal colour factor *A* and the factor for self-colouring *B* as well as the intensifier *C*, which, on the other hand, were present in Canadian Wonder. The cross was therefore *AABBCCRRss* × *aabbccRRSS*, and the F_1 was *AaBbCcRRSs*, in other terms: coloured-seeded (*A*), mottled (*Bb*), vivid colour and brown umbilical ring (*C*), wine-red (*R*), striped (*S*). In this type of colouring the mottling is very dark and makes the striping almost invisible. Eliminating the factors that segregate freely we find that in this case all gametes have *R*, but that *B* and *S*, coming from different partners, must

[1] In this fifth article the white seeded forms are said to lack *S*. This must apparently be corrected, as already stated in the 9th article. According to our present knowledge all forms mentioned in the 5th article possess (*R* and) *S*, causing also pale red pod striping. The white seeded ones are *aa*, the coloured ones *AA* or *Aa*. The difference between pale red, greyish blue etc. and the full seed- and pod-colours is probably caused by different states of the intensifying factor *C*.

repulse each other. The great majority of gametes are therefore *BRs* and *bRS*. The F_2 will practically consist of *BBRRss*, *bbRRSS* and *BbRRSs*. In all these three classes $^1/_4$ will be white seeded (*aa*), and of the coloured forms $^1/_4$ is *cc* and shows a paler red. Discarding the white seeded plants I found in the F_2 of this cross the following figures: out of 41 plants 10 had self-coloured seeds (exp. 10,25), 22 dark mottled/striped (exp. 20,50) and 9 normally striped (exp. 10,25). In the F_3, which was raised from all the 41 F_2-plants, 30 series segregated white seeded plants. Apart from these the self-coloured gave only self-coloured, the striped only striped and the mottled types gave all three classes. No cross-overs were found in this experiment, but the numbers of plants in the F_3-series were rather small. Owing to the *cc*-condition of the one partner a certain number of plants showed a paler red.

In a third cross, like the foregoing preliminarily mentioned in KOOIMAN's and my 9th article (1923), Canadian Wonder was combined with another white form that was known to possess the factor *Bl* that changes the red caused by *R* to blueish black. In respect of other factors this white seeded strain was like No. 13. The results of the cross are comparable with those of the foregoing with the only difference that $^3/_4$ of the coloured forms have black instead of red and that $^2/_3$ of those black seeded forms (or grey when *cc*) segregate in black and red. Apart from white and neglecting the difference between black and red the actual figures were: in the F_2 out of 28 plants 6 self-coloured, 14 mottled, 8 striped; in the F_3 the self-coloured and the striped all constant, the mottled all segregating.

The fourth cross was one between »Hinrich's Giant» and a buff seeded variety. Hinrich's Giant has pale red striped seeds without umbilical ring. Its formula is *AAbbccRRSS*. The buff type was a segregate from the cross described and analyzed in KOOIMAN's dissertation (1922). Its set of factors was the same as that of the brown bean with the exception of the intensifier *C*, :*AABBccDDrrss*. The result was as expected: an F_1 with mottled buff seeds, that also showed a somewhat light red striping and an F_2 consisting of self-coloured, mottled-striped and striped in a 1 : 2 : 1 proportion. The complication caused by *D* made that amongst the coloured types $^1/_4$ had pale yellow as basal colour instead of buff and lacked the brown umbilical ring. The actual numbers were: out of 76 plants 15 buff self, 6 yellow self, 14 striped darker, 4 striped paler, 28 mottled-striped on buff, 8 the same on yellow. Apart from these I found one plant with yellow mottled seeds without any trace of striping, apparently a cross-over of the *Bbrrss*-type, from ga-

metes *brs* and *Brs*. The 35 seeds of this plant had no umbilical ring and gave a normal next generation of 8 yellow self, 17 mottled yellow and 7 white. It is very probable that also some *BRS*-gametes have been formed in the F_1, but *BBRRSS* cannot be distinguished from *BBRrSs* unless by further experiments. Cross-overs of the *RRss*-type (self-coloured red) did not appear.

The fifth cross was one between the same buff variety as used in the preceding experiment and the striped red Dutch »Lapwing's Egg» variety used in the first cross: *AABBccDDrrss* × *AAbbCCDDRRSS*. The result was about the same as in the first cross, the only difference being the condition of *C*, but in this cross three cross-overs were noted. In addition to the types expected: *BBrrss* (self-coloured, orange-brown with *CC* or *Cc*, buff with *cc*), *BbRrSs* (darker or lighter mottled with red stripes) and *bbRRSS* (red striped on darker or lighter bottom), two plants with mottled buff seeds without stripes and one with self-coloured red seeds were met with in the F_2. These plants must owe their existence to the formation of cross-over gametes, the mottled buff of *brs*, the self-coloured red of a *BRs*-gamete. More cross-over gametes and -individuals of other genotypes may have occurred but could not be recognized.

The number of recognizable cross-overs is, as appears from the foregoing, very low. In the whole material of the experiments mentioned, including some F_3-generations comprising about 6500 plants, only five *B—RS* and four *R—S* breaks have been found. Even if the non-recognizable cross-overs have occurred twice as often the whole amount of crossing-over is less than 1 %. All three factors *B*, *R* and *S* must therefore be supposed to lie very close together on their chromosome.

II. THE C—G LINKAGE.

In the course of my experiments on seed coat factors a cross was made between »Sutton's Prolific» (Pr.), a buff seeded variety, and an orange-brown seeded strain received from Professor W. JOHANNSEN as a »pure line». The latter will in the following be called »Y».

From earlier work it was known that these two varieties differed, regarding seed coat colour, in one factor only. This factor must be *C*, as a difference in *B* would have caused mottling in the heterozygotes and no mottled beans did occur in the progeny of the cross. Apart from the difference in seed colour the two varieties differ also in a number of other characters that will not be spoken of in this paper. There is

a slight difference in the colour of the half-ripe pod, however, that must be mentioned. Both strains have medium green pods, but in Y the green colour is a shade lighter than in ordinary green podded varieties, while Pr. has a shade of yellow in its half-ripe pods.

The F_1 of this cross consisted of two plants only and one of them was destroyed at an early stage. The other became a very large, richly flowering, bushy plant with normally green leaves and pods. It gave about 150 seeds after isolation in a muslin cage. The F_2 numbered 136 plants. Most unexpectedly 38 of them had yellow pods as wax beans. This is an approximative, rather good 1 : 3 proportion, but the distribution of the yellow and green pods was not the same in the orange-brown seeded and the buff seeded groups. The F_3 was grown in the course of three successive years in three series of 50 seeds sown each year. The results were almost identical every year:

	1st series		*2nd series*		*3rd series*		*the whole F_2*		
	green pods	yellow pods	green pods	yellow pods	green pods	yellow pods	green pods	yellow pods	Total
ora.-br. seeds	24	7	25	9	27	8	76	24	100
buff seeds	8	5	6	4	8	5	22	14	36
							98	38	136

The figures suggest a feeble linkage between the two factors that cause the differences in seed and in pod colour.

In order to calculate the cross-over percentage we must correct the figures somewhat. After this correction (according to the method described by WINGE 1928) the F_2-figures are:

ora.-br. seeds:	green pods	94,48	yellow pods	25,92	together 120,16
buff seeds:	» »	25,59	» »	14,00	» 39,52
together:	» »	120,07	» »	39,92	159,99

From these corrected figures a cross-over percentage = 40,35 % may be calculated. The figures actually found in the experiment, however, correspond more closely with a gametic ratio of 2 : 1 than with a ratio of 1,5 : 1 (as would be in accord with 40 % crossing-over). If the gametic ratio is about 2 : 1 the segregation would be according to a 4 : 5 : 5 : 22 scheme, which nearly covers the facts. The conclusion is drawn that the gametal ratio is about 1,8 : 1, with accordingly a cross-over percentage of about 35 %.

The results of the F_3, that was raised from all the 44 F_2-individuals, agree completely with the hypothesis of linkage between the two factors.

In an Appendix to this paper the actual figures are given and compared with the expected proportions on a gametal ratio 2 : 1.

As to the difference between green and yellow podded beans there is no reason to disagree with EMERSON's interpretation, according to which there is a triple allelomorphic series, the highest term causing green pods (and foliage), the middle term green foliage but yellow pods, and the lowest term yellow leaves and pods. I call these allelomorphs *Gr*, *G* and *g* respectively, and we have seen that this *G*-locus lies on the same chromosome as *B*. The lowest term *g* does not occur in the present material, only gametes with *Gr* and *G* having been in action.

The question arises, why the one parent form has green pods while it in the cross behaves as a yellow podded variety; in other words: how to interpret the existence of *G*-gametes, where both parents have green pods?

As the F_2 was built up on one F_1-plant only I at first considered the possibility of an experimental error. But the other characters (flower colour, pod and seed shape and length, seed colour) of the parent varieties reappear and segregate normally in the hybrids. Moreover, at the time the cross was made and studied no wax beans were grown in my experiment garden or in the neighbourhood.

Having tried many hypotheses I think the only plausible interpretation is that the Pr.-parent plant has been a heterozygous *GrG*-individual.

In another buff seeded race we (TJEBBES & KOOIMAN III, 1919) found a constant and rather considerable percentage of *Grg*-heterozygotes that threw 25 % yellowish white (*gg*) seedlings. Such seedlings die at an early stage of life. Considering that Pr. is a commercial race we must admit the possibility that among the great majority of *GrGr*-individuals now and then some rare *GrG*-plants occur. I have no record of yellow podded *GG*-segregates from such plants ever having been found in large crops of the variety, but as such plants would be recognizable only during the short later middle part of the ripening period (Pr. is an extraordinarily early bean), when many of the surrounding green podded plants already have ripe or nearly ripe pods (which are yellowish brown), they may easily have been overlooked. Moreover the *GG*-plants, although fully viable, are considerably weaker than the green podded ones.

The Pr.-parent of my cross may therefore well have been a *GrG*-individual and the F_1-plant may have arisen from the combination of a *G*-gamete produced by it with a normal *Gr*-gamete from the Y.-parent.

SUMMARY.

The factors *B*, *C*, *G*, *R* and *S* are described and shown to form two linkage groups. *B*, *R* and *S* lie on the same chromosome; their linkage is very close (less than 1 % crossing-over). *C* and *G* lie on another chromosome; their linkage is rather loose, with a cross-over percentage of about 35 %.

APPENDIX. *F_3 of the cross between* SUTTON's *Prolific and Prof.* JOHANNSEN's *Y-strain.*

The F_1 was *CcGrG*. *CGr-*, *CG-*, *cGr-* and *cG*-gametes are supposed to be produced in a proportion of about 2 : 1 : 1 : 2. *C* (brown seed) dominant over *c* (buff seed) and *Gr* (green pod) dominant over *G* (yellow pod). In the F_2 the four combinations were represented with 5, 7, 8 and 24 individuals respectively. (Exp. 4,89; 6,11; 6,11; 26,89).

F_2-class	F_2-field-number	F_3-classes and numbers of plants				Expectation
		green/brown	green/buff	yellow/brown	yellow/buff	
yellow/buff	83	0	0	0	27	All constant yellow/buff (*ccGG*)
»	84	0	0	0	25	
»	85	0	0	0	5	
»	86	0	0	0	18	
»	87	0	0	0	28	
»		0	0	0	103	
yellow/brown *Ia*	88	12	5	4	3	Five classes: *Ia* (*CcGrG*) segregating four types 22:5:5:4 *Ib* (*CcGrG*) segregating four types 19:8:8:1 *II* (*CcGrGr*) constant green pod, segreg. brown and buff seed 3:1 *III* (*CCGrG*) constant brown seed, segreg. green and yellow pod 3:1 *IV* (*CCGrGr*) constant. Class ratio: Ia:Ib:II:III:IV 8:2:4:4:4
» *Ia*	89	12	3	4	2	
» *Ia*	90	14	2	2	2	
» *II*	91	26	3	0	0	
» *IV*	92	25	0	0	0	
» *IV*	93	29	0	0	0	
» *Ia*	94	16	5	3	2	
» *IV*	95	26	0	0	0	
» *II*	96	17	6	0	0	
» *III*	97	23	0	6	0	
» *III*	98	19	0	7	0	
» *Ia*	99	13	2	3	1	
» *III*	100	19	0	6	0	
» *II*	101	21	8	0	0	
» *Ib*	102	16	6	4	0	
» *III*	103	17	0	7	0	
» *Ib*	104	12	6	6	1	
» *II*	105	20	8	0	0	
» *III*	106	23	0	7	0	
» *Ib*	107	14	6	7	1	
» *Ia*	108	11	2	4	2	
» *Ia*	109	18	3	3	3	
» *II*	110	19	7	0	0	
» *Ia*	111	15	4	5	4	
» *Ia* (8numb.)		111	26	28	19	
» *Ib* (3 »)		42	18	17	2	
» *II* (5 »)		103	32	0	0	
» *III* (5 »)		101	0	35	0	
» *IV* (3 »)		80	0	0	0	

F_2-class	F_2-field-number	F_2-classes and numbers of plants				Expectation
		green/brown	green/buff	yellow/brown	yellow/buff	
yellow/brown	112	0	0	16	4	Two classes: a) constant (*CCGG*), b) segreg. brown and buff seed 3 : 1 (*CcGG*) Class ratio: const. : segr. 1 : 4
»	113	0	0	16	6	
»	114	0	0	14	5	
»	115	0	0	22	5	
»	116 ×	0	0	25	0	
»	117 |	0	0	20	6	
»	118 ×	0	0	27	0	
»	119 |	0	0	20	8	
» const. numb.	(2)	0	0	52	0	
» segreg. »	(6)	0	0	108	34	
green/buff	120 ×	0	22	0	0	Two classes: a) constant (*ccGrGr*), b) segreg. green and yellow pod 3 : 1 (*ccGrG*) Class ratio: const. : segr. 1 : 4
»	121 |	0	14	0	10	
»	122 |	0	18	0	8	
»	123 |	0	24	0	6	
»	124 |	0	16	0	3	
»	124 |	0	20	0	5	
»	126 ×	0	30	0	0	
» const. numb.	(2)	0	52	0	0	
» segreg. »	(5)	0	92	0	32	

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GELBROSTBESTIMMUNGEN AN WINTERGERSTE

EINE STATISTISCH-METHODOLOGISCHE UNTERSUCHUNG

VON *S. WAHLUND*
STAATSINSTITUT FÜR RASSENBIOLOGIE, UPPSALA

FOLGENDER Aufsatz bringt die Ergebnisse gewisser, im Sommer 1930 in Svalöf ausgeführter Gelbrostbestimmungen (*Puccinia glumarum*) an Wintergerste. Das Material wurde mir von Herrn Professor H. NILSSON-EHLE in liebenswürdiger Weise zur Verfügung gestellt. Der Zweck dieser Studie besteht in erster Linie darin, eine geeignete statistische Methodik für derartige Untersuchungen zu demonstrieren.

Die Pflanzen wurden nach der Ernte untersucht und zwar in der Weise, dass bei jeder Pflanze die Menge von Teleutosporen an den Blattscheiden geschätzt wurde.

Alles in allem wurden untersucht: 1877 Pflanzen von »Derenburg 8», 1557 Pflanzen von »Janetzki Original», 1499 Pflanzen von »Mansholt, Groningen, Original», und 1586 Pflanzen von »Eckendorfer Original». Sämtliche Sorten waren auf je drei Parzellen (I, II, III; siehe Tab. 1) verteilt.

Die Rostbestimmungen wurden von sechs Untersuchern ausgeführt, die paarweise arbeiteten. Jedes Paar (I, II bezw. III) untersuchte eine Parzelle von jeder der vier Sorten (eine »Seriengruppe»).

Zu Beginn der Untersuchung wurden je nach der Stärke des Rostangriffes vier Grenzen festgestellt, nämlich derart, dass Grenze 1 einen sehr schwachen Rostangriff, Grenze 2 einen ziemlich schwachen, Grenze 3 einen ziemlich starken und Grenze 4 einen sehr starken Rostangriff markierte. Die Grenzen wurden auf Grund einer Besichtigung des untersuchten Materials gezogen. Diesen Grenzen entsprechend erfolgte eine Aufteilung des Materials in fünf Gruppen (siehe Tab. 1).

Bei dieser Gruppierung achtete man sorgfältig darauf, dass die Gruppengrenzen im ganzen Materiale einheitlich gezogen wurden. Da dabei Pflanze mit Pflanze auf dem Versuchstische verglichen wurde, besteht eine gute Gewähr dafür, dass es auch gelungen sei, eine ein-

TABELLE 1. *Rostbestimmungen an Wintergerste.*

a) *Absolute Zahlen.*

Serien-gruppe	Nummer von Svalöf, Jahr 1930[1]	Anzahl der Bestimmungen, die zwischen folgende Grenzen fielen					Summe
		0—1	1—2	2—3	3—4	4—5	
I	2	500	95	54	5	—	654
	3	7	68	267	60	21	423
	4	86	254	145	42	5	532
	5	168	298	120	6	—	592
II	2	424	117	20	—	—	561
	3	—	28	218	210	31	487
	4	58	173	140	32	3	406
	5	55	214	97	15	3	384
III	2	440	175	47	—	—	662
	3	14	119	304	201	9	647
	4	249	205	88	19	—	561
	5	139	284	159	28	—	610

b) *Relative Zahlen.*

Serien-gruppe	Nummer von Svalöf, Jahr 1930[1]	Von den Bestimmungen fielen folgenden Anteile (in %) zwischen die Grenzen				
		0—1	1—2	2—3	3—4	4—5
I	2	$76{,}45 \pm 1{,}66$	$14{,}53 \pm 1{,}38$	$8{,}26 \pm 1{,}08$	$0{,}76 \pm 0{,}34$	—
	3	$1{,}65 \pm 0{,}62$	$16{,}08 \pm 1{,}79$	$63{,}12 \pm 2{,}35$	$14{,}18 \pm 1{,}70$	$4{,}96 \pm 1{,}06$
	4	$16{,}17 \pm 1{,}60$	$47{,}74 \pm 2{,}17$	$27{,}26 \pm 1{,}93$	$7{,}89 \pm 1{,}17$	$0{,}94 \pm 0{,}42$
	5	$28{,}38 \pm 1{,}85$	$50{,}34 \pm 2{,}05$	$20{,}27 \pm 1{,}65$	$1{,}01 \pm 0{,}42$	—
II	2	$75{,}58 \pm 1{,}81$	$20{,}86 \pm 1{,}72$	$3{,}57 \pm 0{,}78$	—	—
	3	—	$5{,}75 \pm 1{,}05$	$44{,}76 \pm 2{,}25$	$43{,}12 \pm 2{,}24$	$6{,}37 \pm 1{,}11$
	4	$14{,}29 \pm 1{,}74$	$42{,}61 \pm 2{,}45$	$34{,}48 \pm 2{,}36$	$7{,}88 \pm 1{,}34$	$0{,}74 \pm 0{,}40$
	5	$14{,}32 \pm 1{,}79$	$55{,}73 \pm 2{,}53$	$25{,}26 \pm 2{,}22$	$3{,}91 \pm 0{,}99$	$0{,}78 \pm 0{,}46$
III	2	$66{,}47 \pm 1{,}83$	$26{,}44 \pm 1{,}71$	$7{,}10 \pm 1{,}00$	—	—
	3	$2{,}16 \pm 0{,}57$	$18{,}39 \pm 1{,}15$	$46{,}99 \pm 1{,}96$	$31{,}07 \pm 1{,}82$	$1{,}39 \pm 0{,}46$
	4	$44{,}39 \pm 2{,}10$	$36{,}54 \pm 2{,}03$	$15{,}69 \pm 1{,}53$	$3{,}39 \pm 0{,}76$	—
	5	$22{,}79 \pm 1{,}70$	$46{,}56 \pm 2{,}02$	$26{,}07 \pm 1{,}78$	$4{,}59 \pm 0{,}85$	—
Standardserie (I: 4 + II: 4)		$15{,}35 \pm 1{,}18$	$45{,}52 \pm 1{,}63$	$30{,}38 \pm 1{,}50$	$7{,}89 \pm 0{,}88$	$0{,}85 \pm 0{,}10$

[1] Bedeutung der Ziffern: 2 = Derenburg 8; 3 = Janetzki Original; 4 = Mansholt, Groningen, Original; 5 = Eckendorfer Original.

heitliche Bestimmungstechnik durchzuführen. Möglicherweise könnte Anlass zu dem Verdachte vorliegen, dass trotz des Zusammenarbeitens der drei Gruppen von Untersuchern gewisse Unterschiede in der Bestimmungstechnik zwischen den Seriengruppen — wenn auch nicht innerhalb der Seriengruppen — aufgetreten sein dürften. Dies würde aber bei den im folgenden zur statistischen Analyse der Resultate angewandten Methoden keine entscheidende Rolle spielen. Irgendwelche Tatsachen, die einen solchen Verdacht stützen können, waren jedoch nicht zu beobachten.

Tab. 1 zeigt, wie die vier untersuchten Sorten in den drei Seriengruppen sich nach der Stärke des Rostangriffes verteilen [1].

Vergleicht man in Tab. 1 b die prozentuelle Verteilung der vier Sorten, so treten deutliche, sichergestellte Unterschiede zu Tage. Sowohl wenn man die Sorten innerhalb einer Seriengruppe oder unabhängig von den Seriengruppen miteinander vergleicht, so zeigt es sich, dass Derenburg 8 durchwegs beträchtlich weniger vom Roste angegriffen ist als Mansholt, Groningen, Original und Eckendorfer Original sowie dass diese beiden Sorten viel weniger als Janetzki Original vom Roste angegriffen sind. Auch zwischen den beiden mittleren Sorten zeigen sich völlig gesicherte, aber nicht eindeutige Unterschiede.

Vergleicht man dieselbe Sorte in den drei Seriengruppen, so ergeben sich auch hier sichergestellte, aber im grossen und ganzen nicht so ausgeprägte Unterschiede. Deutlichere Differenzen treten vor allem bei Janetzki Original und Mansholt, Groningen, Original, auf. Die erste Sorte zeigt in der Seriengruppe II einen grösseren Rostangriff als in den beiden übrigen Seriengruppen; die zweite Sorte aber weist in der Seriengruppe III einen beträchtlich geringeren Rostangriff als in den beiden übrigen Seriengruppen auf.

Aus einer Verteilungstabelle dieser Art kann man jedoch nur eine grobe Orientierung über die Lage erhalten. Man bekommt keine einheitlichen Ausdrücke für die Grössenordnung der Abweichungen zwischen den verschiedenen Serien, was jedoch für die Beurteilung des erhaltenen Resultates nötig ist.

Wäre es versuchstechnisch gänzlich gelungen gewesen, in den verschiedenen Parzellen dasselbe Milieu zu schaffen, und wäre (was jedoch zu vermuten ist) die Einteilungsnorm, derzufolge das Material in unsere fünf Klassen geschieden wurde, völlig einheitlich, so könnte man

[1] Die Tabellen stellen das Material in fünf Gruppen nach ansteigenden Graden der Stärke des Rostangriffes dar, also nach steigender Rostempfänglichkeit und nicht, wie es üblich sein dürfte, nach steigender Rostresistenz.

unmittelbar vollkommen sicher sein, dass die entstandenen Differenzen zwischen den untersuchten Sorten tatsächlich vorhandene Unterschiede der Rostresistenz wiederspiegelten. Unter solchen Verhältnissen könnten jedoch keine sichergestellten Abweichungen zu bemerken sein, wenn man dieselbe Sorte von Seriengruppe zu Seriengruppe vergleicht.

Wie wir gesehen haben, entstehen jedoch auch hier Differenzen, woraus hervorgeht, dass die Milieuverhältnisse in den verschiedenen Parzellen (und eventuell auch die Gradierungstechnik) verschiedenartig gewesen sind. Es handelt sich nun darum, ob diese Unterschiede sich in so geringem Grade geltend machen, dass der Beweiswert der zwischen den verschiedenen Sorten entstandenen Differenzen nicht zweifelhaft wird, mit andern Worten, ob die Abweichungen, die beim Vergleich derselben Sorte in verschiedenen Bestimmungsserien zu Tage treten, hinlänglich klein sind, wenn man sie mit den Abweichungen zwischen verschiedenen Sorten vergleicht. Um dies zu untersuchen, brauchen wir einheitliche Ausdrücke der Stärke des Rostangriffes in den verschiedenen Serien.

Wenn man bei der Pflanzenveredlung einen einheitlichen Ausdruck für Rostempfänglichkeit und ähnliche variable Eigenschaften, die sich quantitativ nicht messen lassen, darstellen will, so pflegt man oft auf folgende Weise zu verfahren[1]. Nachdem man die Verteilung eines untersuchten Attributes nach Gruppen (Graden) der untersuchten Eigenschaft festgestellt hat, legt man den verschiedenen Gruppen gewisse Werte bei, die der Reihe nach gewöhnlich den Gliedern der arithmetischen Serien 1, 2, 3, 4 u. s. w. oder 0,5, 1,5, 2,5, 3,5, u. s. w. entsprechen. Dann berechnet man Mittelwerte, Standardabweichung und mittlere Fehler derart, als ob man es mit einer (heterograden) Serie nach einer quantitativen Eigenschaft mit obengenannten Werten als Klassenmittelwerten zu tun hätte. Würde man nach üblicher Methode den Mittelwert einer gewissen Serie in Tab. 1 berechnen, so würde zum Beispiel die Anzahl in Gruppe 0—1 mit 0,5, die in 1—2 mit 1,5 u. s. w. und schliesslich die in Gruppe 4—5 mit 4,5 multipliziert werden, worauf die Summe der erhaltenen Zahlen durch die Gesamtzahl der Serie dividiert werden würde [z. B. für die Serie I: $2,\frac{1}{654}\cdot(500\cdot 0,5+95\cdot 1,5+54\cdot 2,5+5\cdot 3,5)$]. Der Grad der mittleren Empfänglichkeit M würde sich also aus folgender Formel ergeben:

[1] Auch in verschiedenen andern Forschungszweigen, z. B. in der Anthropologie, wird diese statistische Methode angewendet. Vgl. MARTIN 1928, S. 82.

$$M = \sum \frac{n_i}{n} \cdot (i + 0{,}5) \quad \ldots\ldots\ldots\ldots (1),$$

[vgl. Formel (6)], wo n_i die Anzahl Bestimmungen bezeichnet, die zwischen den Grenzen i und $i + 1$ liegen, und wo n die Gesamtzahl der Bestimmungen ausdrückt. Den mittleren Fehler $\varepsilon(M)$ von M erhält man nach derselben Methodik aus der Formel:

$$\varepsilon(M) = \sqrt{\frac{\sum \frac{n_i}{n} \cdot (i + 0{,}5 - M)^2}{n}} \quad \ldots\ldots\ldots (2).$$

Man kann die Sache derart ausdrücken, dass man an den einzelnen Pflanzen mittels einer fünfgradigen Skala eine Gradierung nach der Stärke des Rostangriffes durchführt. Diejenigen, welche unter die Grenze 1 fallen, erhalten den Grad 0,5, diejenigen zwischen den Grenzen 1 und 2 den Grad 1,5 u. s. w. Hierauf berechnet man den mittleren Grad [Formel (1)]. Stellen nun die auf diese Weise berechneten mittleren Grade der verschiedenen Serien zuverlässige Masse der Rostempfänglichkeit dar? Die Frage kann keineswegs bedingungslos in bejahendem Sinne beantwortet werden. Der berechnete mittlere Grad wird nämlich nicht nur von der Rostempfänglichkeit, sondern auch von der Norm der Gradierung beeinflusst. Beim Vergleiche dieser mittleren Grade verschiedener Sorten können die Resultate bei verschiedenen Gradierungsprinzipen verschieden ausfallen. Nimmt man an, die Empfänglichkeit für Rost sei durch ein quantitatives Mass ausdrückbar, so würde der Vergleich erst dann (im Verhältnis zu diesem Masse) korrekte Resultate ergeben, wenn die Gruppenmittelwerte (die mittlere Empfänglichkeit der Gruppen), in dem Masse ausgedrückt (sowie die bei der Gradierung angewandten Werte 0,5, 1,5, 2,5 u. s. w.) eine arithmetische Serie bildeten, d. h., wenn die Entfernungen zwischen den Mittelwerten zweier aufeinanderfolgender Gruppen durchgehend gleich gross sind. Unter der Voraussetzung, dass die Klasseneinteilung genügend differenziert ist, kann die Sache derart ausgedrückt werden, dass ein nach Formel (1) berechneter Mittelwert erst dann völlig korrekt wird, wenn die Klassenbreiten (Grade) gleich gross sind. Nun ist es natürlich unmöglich, für eine quantitativ nicht messbare, variable Eigenschaft direkt folgerichtig eine Gruppeneinteilung (Gradierung), die dieser Bedingung entspricht, durchzuführen. Die Grenzen zwischen den verschiedenen Gruppen (Graden) müssen stets mehr oder weniger subjektiv gezogen

werden. Einige Gruppen (Grade) werden ein grösseres Intervall umfassen, andere ein kleineres.

Misst man also die Rostempfänglichkeit mit einer Skala, auf der die Entfernungen zwischen den Graden der Skala verschieden gross sind, so kann man natürlich im grossen und ganzen zu richtigen Ergebnissen kommen, allerdings nicht mit Sicherheit. Um die Sache klarer zu fassen, denken wir uns, dass wir anstatt der Rostempfänglichkeit die Pflanzenhöhe verschiedener Sorten zu messen haben und dazu ein Metermass anwenden, bei dem die Entfernungen zwischen den »Zentimeterstrichen« ungleich gross sind. Wenn eine Sorte in Wirklichkeit grössere mittlere Höhe als eine andere hat, so ist es freilich, besonders wenn die Höhendifferenz gross ist, in gewissem Grade wahrscheinlich, dass die auf Grund der Messungen mit dem mangelhaften Metermasse berechnete mittlere Höhe der ersteren Sorte grösser ist als die der letzteren, aber es ist keineswegs sicher, dass dies der Fall sein werde. Unter allen Verhältnissen ergeben die berechneten mittleren Höhen schlechte Masse betreffend das Grössenverhältnis der Sorten untereinander. Es ist selbstverständlich, dass, je mehr die Entfernungen zwischen den »Zentimeterstrichen« des angewendeten Metermasses differieren, in der Regel das erhaltene Resultat umso unsicherer wird.

Analoge Verhältnisse gelten natürlich, wenn man auf die oben erwähnte Weise den Mittelwert der Rostempfänglichkeit oder andere nicht quantitativ messbare Eigenschaften berechnet. Es dürfte überflüssig sein, die Begrenzung der Methode zu präzisieren und zu erörtern, unter welchen Bedingungen sie zu zufriedenstellenden Resultaten führt, insbesondere da sie im folgenden durch eine andere ersetzt werden wird, der die oben berührten Mängel der ersteren nicht anhaften. Soviel kann man jedoch sagen, dass die mit Hilfe von Formel (1) berechneten Mittelwerte, unabhängig von der Klasseneinteilung, zwar keine zufriedenstellenden Angaben über die Grösse der Differenzen zwischen gewissen untersuchten Attributen liefern, dass sich aber aus jenen Mittelwerten (bei einem hinlänglich grossen Material) die Rangordnung zwischen den Attributen ergibt unter der Voraussetzung, dass deren Standardabweichung in bezug auf die untersuchte Eigenschaft dieselbe und die Verteilung normal ist. Auch wenn man meint, von der Giltigkeit der genannten Voraussetzung ausgehen zu können, so kann man jedoch nicht sicher sein, dass man die Differenzen der Mittelwerte korrekt beurteilt, da die Grösse der nach Formel (2) berechneten mittleren Fehler natürlich so wie die Mittelwerte von der angewendeten Gradierungsnorm beeinflusst wird.

Wie soll man dann von der Einteilungsnorm unabhängige einheitliche Ausdrücke für die Rostempfänglichkeit verschiedener Sorten herstellen, wenn man deren Verteilung (Tab. 1) auf Klassen kennt, deren Mittelwerte unbekannt sind und welche Klassen keineswegs a priori als gleich gross angenommen werden können? Mehrere Methoden sind hier denkbar. Die Methode, die im folgenden zur Anwendung kommt, wurde sowohl auf Grund der relativen Einfachkeit ihrer theoretischen Unterlage (was nicht zu unterschätzen ist, da sie dadurch auch Forschern ohne höhere mathematische Schulung zugänglich wird) als auch wegen ihrer leichten praktischen Anwendbarkeit gewählt.

Wir gehen zu dem Beispiele der Pflanzenhöhe zurück, welche mit dem mangelhaften Metermasse gemessen wurde. Man nehme an, dass man erst nach Ausführung der Messungen entdecke, dass die Entfernungen zwischen den »Zentimeterstrichen« des Metermasses ungleich gross sind. Man würde da den Schaden dadurch gutmachen können, dass man das exakte Mass, welches jedem »Zentimeterstriche« des mangelhaften Massstabes entspricht, bestimmt und die derart erhaltenen Werte den Berechnungen zugrunde legt. Wenn eine Pflanze nach dem mangelhaften Massstabe eine gewisse Zahl von »cm« misst, so wird also diese Zahl bei den Berechnungen der Mittelwerte nicht angewendet, sondern dasjenige Mass, welches den betreffenden »cm« auf dem exakten Metermasse entspricht.

Auf dieselbe Weise muss bei der Berechnung des Mittelwertes der Serien in Tab. 1 verfahren werden. Eine Pflanze, die z. B. der Gruppe 2—3 zugeteilt worden ist, soll bei der Berechnung der Mittelwerte nicht mit dem Werte 2,5 nach Formel (1) einbezogen werden, sondern mit einem Werte, der soviel wie möglich den »wirklichen» Klassenmittelwert der Gruppe 2—3 ausdrückt.

Es handelt sich also darum, Ausdrücke für die Klassenmittel der verschiedenen Gruppen 0—1, 1—2, ..., 4—5 zu schaffen, um in der Formel (1) die willkürlich gewählten Werte, 0,5, 1,5, ..., 4,5, zu ersetzen.

Um die verschiedenen Klassenmittelwerte zu finden, benötigen wir teils einen fixen Punkt, von dem aus die Masse gemessen werden — einen Orientierungspunkt — teils eine Masseinheit, in der die Masse ausgedrückt werden. Wie dieser Orientierungspunkt und diese Masseinheit gewählt werden, ist selbstverständlich prinzipiell gleichgültig. Als Orientierungspunkt kann eine unserer Klassengrenzen (1, 2, 3, bezw. 4) und als Masseinheit eine unserer Klassenbreiten (1—2, 2—3 bezw. 3—4) angewendet werden. Man kann auch als Orientierungspunkt den Mittelwert und als Masseinheit die Standardabweichung

einer unserer Serien (eventuell die Summe zweier oder mehrerer Serien) anwenden. PEARSON (1906), der das Problem, quantitative Klassenmittelwerte aus ähnlichen Verteilungen wie in Tab. 1 zu bilden, behandelt hat, führt die beiden Methoden an, wählt aber die erstere auf Grund der verschiedenartigen Variabilität in den von ihm untersuchten Serien[1]. Dieser Grund, der bei PEARSONs Anwendung der Klassenmittelwerte für die Wahl der Methode entscheidend wurde, spielt jedoch keine prinzipielle Rolle bei der im folgenden vorgeschlagenen Methode. Dass wir hier eine Standardserie gewählt und ihre Standardabweichung als Masseinheit sowie ihren Mittelwert als Orientierungspunkt festgesetzt haben, geschah teils deswegen, weil dadurch die Berechnungen einfacher werden, aber auch deshalb, weil es beim Vergleich mit nach anderer Gradierungstechnik gebildeten Bestimmungsserien einen gewissen Vorteil bedeuten kann, dass die dargestellten Werte in Charakteristiken einer bestimmten untersuchten Sorte ausgedrückt werden und also nicht auf die bei einer vorliegenden Untersuchung angewendete Gradierungstechnik zurückzuführen sind.

Wir haben also für eine gewisse gewählte Standardserie ihre Klassenmittelwerte mit dem Mittelwerte der Serie als Orientierungspunkt ($M = 0$) und ihrer Standardabweichung als Masseinheit ($\sigma = 1$) zu bestimmen, d. h. es handelt sich um die Bestimmung der Entfernung der Klassenmittelwerte vom Mittelwert, ausgedrückt durch die Standardabweichung. Um dies ausführen zu können, muss man betreffs der Verteilung der Serie eine Annahme machen. A priori wissen wir streng genommen nicht mehr, als dass eine »höhere» Gruppe der Serie einen höheren, eine »niedrigere» Gruppe einen niedrigeren Klassenmittelwert aufweist. Wenn man annimmt, dass die Standardserie normal verteilt ist (nach einem hypothetischen, quantitativen Mass) und somit ihre relative Verteilung mit gewähltem Orientierungspunkt und Masseinheit graphisch repräsentiert wird durch die bekannte normale Häufigkeitskurve

$$y = \frac{1}{\sqrt{2\pi}} \cdot e^{-\frac{x^2}{2}} \quad \ldots\ldots\ldots\ldots\ldots (3),$$

(vgl. das Diagramm), so dürfte wohl die Annahme in den meisten Fällen — man kann ja mit Rücksicht darauf eine geeignete Standardserie

[1] CHARLIER (1920) gibt eine geeignete Methode für Berechnung des Mittelwertes und der Standardabweichung mit einer Klassengrenze als Orientierungspunkt und einer Klassenbreite als Masseinheit in dem Falle, wo drei Klassen vorhanden sind.

wählen — mit hinlänglicher Genauigkeit den tatsächlichen Verhältnissen entsprechen.

Man nehme nun an, dass gewisse untersuchte Serien durch $n-1$ Klassengrenzen auf n Gruppen (in vorliegender Untersuchung auf 5 Gruppen) verteilt sind. Es wird angenommen, dass die n Gruppen einer gewissen gewählten Standardserie die Frequenzen $p_1, p_2, \ldots, p_i, \ldots$ bezw. p_n ($\Sigma p_i = 1$) haben. Um die Klassenmittelwerte der Serie zu bestimmen, nehmen wir eine willkürlich gewählte Gruppe, die i-te, also die Gruppe, welche zwischen der $(i-1)$-sten und der i-ten Klassengrenze liegt. Den Teil des Materiales, der unter die $(i-1)$-ste Klassengrenze fällt, bezeichnen wir als P_{i-1}, und den Teil, welcher unter die i-te Klassengrenze zu liegen kommt, als P_i ($P_{i-1} + p_i = P_i$). Wenn die Abszissen der beiden Klassengrenzen mit x_{i-1} bezw. x_i bezeichnet werden, so lassen sich diese aus P_{i-1} bezw. P_i bestimmen; vgl. die Formeln (4).

$$\frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x_i - 1} e^{-\frac{x^2}{2}} dx = P_{i-1}$$

$$\frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x_i} e^{-\frac{x^2}{2}} dx = P_i \quad \ldots\ldots\ldots\ldots \quad (4)$$

Die den Abszissen x_{i-1} und x_i entsprechenden Ordinaten y_{i-1} bezw. y_i der $(i-1)$-sten und der i-ten Klassengrenze können nun durch Formel (3) bestimmt werden.

Die Formeln (3) und (4) wurden nur mitgeteilt, um zusammen mit dem Diagramme diejenigen Prinzipien zu ergeben, nach denen y_{i-1} und y_i berechnet wurden. Bei der praktischen Rechenarbeit sollen Tabellen angewendet werden [PEARSON (1921); PEARSON (1914), Tab. III; SHEPPARD (1903), Tab. IV], aus denen man, wenn man P_{i-1} bezw. P_i kennt (nachdem die Werte $2P_{i-1} - 1$ bezw. $2P_i - 1$ ausgerechnet worden sind) unmittelbar die Werte y_{i-1} bezw. y_i erhält.

Wenn man also y_{i-1} und y_i berechnet hat, wird der Klassenmittelwert M_i der i-ten Gruppe nach folgender Formel [siehe z. B. PEARSON (1914), vergl. JAEDERHOLM (1914)] ermittelt:

$$M_i = \frac{1}{p_i \sqrt{2\pi}} \int_{x_i - 1}^{x_i} x\, e^{-\frac{x^2}{2}} dx = \frac{y_{i-1} - y_i}{p_i} \quad \ldots\ldots\ldots \quad (5)$$

Auch für die beiden offenen Gruppen, die erste und die n:te, kann natürlich der Gruppenmittelwert M_1 bezw. M_n nach Formel (5) berechnet werden; man erhält mit den obigen Bezeichnungen

$$M_1 = \frac{0 - y_1}{p_1} = -\frac{y_1}{p_1}$$

$$M_n = \frac{y_{n-1} - 0}{p_n} = \frac{y_{n-1}}{p_n}$$

Wie man die Berechnungen am geeignetsten praktisch ausführt, wird durch folgendes Rechnungsschema [ein ähnliches findet sich bei PEARSON (1921), vgl. PEARSON (1914)] veranschaulicht:

Als Standardserie wurde (siehe unten) die Summe der Serien I: 4 und II: 4 (siehe Tab. 1 b) gewählt.

Die Klassenfrequenzen der Standardserie (p_i) waren:.....		0,1535	0,4552	0,3038	0,0789	0,0085
Die Klassenfrequenzen ergeben summiert (P_i):	(0)	0,1535	0,6087	0,9125	0,9914	(1)
Die Ordinaten (y_i) werden in einer Tabelle (siehe oben) nachgeschlagen:	(0)	0,2367	0,3840	0,1590	0,0233	(0)
Die Ordinaten werden sukzessive subtrahiert ($y_{i-1} - y_i$):...		−0,2367	−0,1473	+0,2250	+0,1357	+0,0233
Durch Division durch die Klassenfrequenzen (p_i) erhält man die Klassenmittelwerte (M_i):		−1,54	−0,32	+0,74	+1,72	+2,74

Wir haben nun unter Voraussetzung normaler Verteilung in unserer Standardserie[1] für die Gruppen die Entfernungen der Klassenmittelwerte vom Mittelwerte berechnet, ausgedrückt in der Standardabweichung, und zwar mit Hilfe von früher (PEARSON, a. a. O.) auf-

[1] Es wird hierbei nur vorausgesetzt, dass die Standardserie (also nicht die übrigen verglichenen Serien) keine allzu grosse Abweichung von der normalen Verteilung aufweist. Die allgemeinen Schlussätze, zu denen wir bei unserer praktischen Anwendung der Methode kommen, würden dieselben geworden sein, wenn wir (eventuell nach erforderlicher Vereinigung der Gruppen) irgendeine andere, beliebige Untersuchungsserie als Standard gewählt hätten. Es bietet ein gewisses Interesse, dies zu konstatieren, besonders deswegen, weil man bei einem Untersuchungsobjekte wie dem hier vorliegenden möglicherweise befürchten kann, dass ein zufällig wirkender Faktor (z. B. die Bodenbeschaffenheit) in gewissem Ausmasse den Charakter der Verteilung einer ganzen Untersuchungsserie verändern könne.

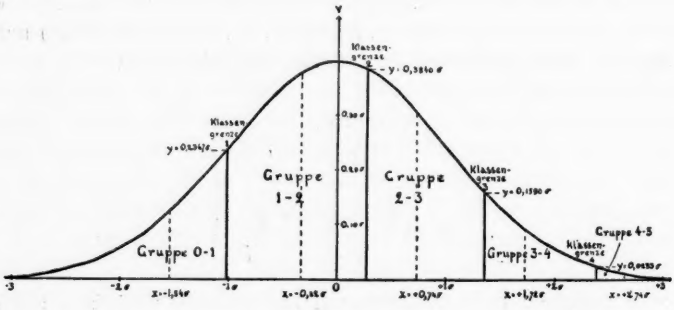

Diagramm. Die Standardserie in eine Normalkurve $y = \frac{1}{\sqrt{2\pi}} \cdot e^{-\frac{x^2}{2}}$ (*σ Masseinheit*) *eingelegt.*

In dem Diagramme sind die Gruppen 0—1, 1—2, 2—3, 3—4, 4—5 derart eingepasst, dass sie 15,35, 45,52, 30,38, 7,89 bezw. 0,85 % der Fläche der Kurve (vgl. Tab. 1 b) einnehmen. Die [z. B. aus der Tabelle in PEARSON (1921) berechnete] Länge der Grenzlinien (die y-Werte) machen 0,2367, 0,3840, 0,1590 bezw. 0,0233 aus. Mit Hilfe dieser Werte werden die Gruppenmittelwerte berechnet, welche — 1,54, — 0,32, + 0,74, + 1,72 bezw. + 2,74 betragen.

gestellten, aber ausserhalb statistischer Fachkreise wenig beachteten statistischen Methoden.

Wir setzen nun unsere Klassenmittelwerte anstatt der willkürlich gewählten Klassenmittelwerte 0,5, 1,5, 2,5 u. s. w. in Formel (1) ein. Den Mittelwert M einer gewissen Serie erhält man somit aus der Formel

$$M = \sum \frac{n_i}{n} \cdot M_i \quad \ldots\ldots\ldots\ldots\ldots \quad (6).$$

Man erkennt nun unmittelbar, dass die aus Formel (6) berechneten Werte die Entfernungen der Mittelwerte der betr. Serien vom Mittelwerte der Standardserie angeben, in deren Standardabweichung ausgedrückt (man rechnet hierbei damit, dass die Klassenmittelwerte der betr. Serien mit denen der Standardserie übereinstimmen).

Für die Serie I: 2 erhält man z. B., wenn I: 4 + II: 4 (siehe Tab. 1 b) zum Standard gewählt wurde, $M = -0{,}7645 \cdot 1{,}54 - 0{,}1453 \cdot 0{,}32 + 0{,}0826 \cdot 0{,}74 + 0{,}0076 \cdot 1{,}72 = -1{,}15$ (vgl. Tab. 2).

Um den mittleren Fehler für M [im folgenden als $\varepsilon(M)$ bezeichnet] zu bestimmen, kann man, wenn die Standardabweichungen der Serien von derjenigen der Standardserie nicht allzu sehr abweichen, für praktische Zwecke die einfache Formel anwenden:

$$\varepsilon(M)=\frac{1}{\sqrt{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (7),$$

wobei n die Gesamtzahl von Einheiten in der Serie bedeutet, von welcher der Mittelwert M gebildet wird.

Die Methode ist zwar grob, bringt aber eine beträchtliche Ersparnis an Rechenarbeit mit sich. Es wäre unnütze Arbeit, hier die üblichen, mehr zeitraubenden Methoden zur Berechnung des mittleren Fehlers anzuwenden. Der mittlere Fehler ist ja hier ein Mass dafür, inwiefern die Abweichungen zwischen den verschiedenen Parzellen unter Voraussetzung des Nichtvorhandenseins von Korrelation zwischen den einzelnen Pflanzen durch den Zufall bedingt sind oder nicht, gibt aber, wie wir im folgenden darlegen, keine definitive Antwort auf die Frage, die man gelöst haben will, nämlich ob die Abweichungen tatsächliche Verschiedenheiten betreffs der Rostresistenz zwischen den untersuchten Sorten widerspiegeln.

Wie schon oben erwähnt, wurde als Standardserie die Summe zweier einander *sehr nahestehender* Bestimmungsserien (I: 4 und II: 4) von der Sorte Mansholt, Groningen, Original gewählt. Zwei Serien wurden verwendet, um die Standardserie ein so grosses Material wie möglich umfassen zu lassen, in der Absicht, die aus der Standardserie berechneten Klassenmittelwerte (M_1) soweit wie möglich von zufälligen Variationen unabhängig zu machen.

Die erhaltenen Mittelwerte [Formel (6)] mit dazugehörigen mittleren Fehlern [Formel (7)] werden in Tab. 2 mitgeteilt.

TABELLE 2. *Entfernung der Mittelwerte der verschiedenen Serien von denjenigen der Standardserie, in deren Standardabweichung ausgedrückt.*

Sorte	Signum	Seriengruppe I	Seriengruppe II	Seriengruppe III
Derenburg 8	2	$-1{,}15 \pm 0{,}039$	$-1{,}21 \pm 0{,}042$	$-1{,}06 \pm 0{,}039$
Janetzki Original	3	$+0{,}77 \pm 0{,}049$	$+1{,}23 \pm 0{,}045$	$+0{,}83 \pm 0{,}039$
Mansholt, Groningen, Original.....	4	$-0{,}04 \pm 0{,}043$	$+0{,}05 \pm 0{,}050$	$-0{,}63 \pm 0{,}042$
Eckendorfer Original	5	$-0{,}43 \pm 0{,}041$	$-0{,}13 \pm 0{,}051$	$-0{,}23 \pm 0{,}040$

Es zeigt sich somit, dass Derenburg 8 in sämtlichen Bestimmungsserien beträchtlich weniger als Janetzki Original angegriffen ist. Der Unterschied zwischen den Mittelwerten der Serien beträgt nicht weniger

als ungefähr 2 Masseinheiten, d. h. die doppelte Standardabweichung der Standardserie (Mansholt, Groningen, Original I und II). Mansholt, Groningen, Original und Eckendorfer Original nehmen eine Mittelstellung ein. Zwischen den drei Bestimmungsserien, die an jeder Sorte ausgeführt wurden, treten zwar völlig sichergestellte Differenzen zu Tage (siehe besonders Janetzki Original, Serie II im Vergleich zu I und III und Mansholt, Groningen, Original, Serie III im Vergleich zu I und II), welche doch im grossen und ganzen nicht derselben Grössenordnung angehören wie diejenigen Differenzen, die zwischen verschiedenen Sorten entstehen.

Wenn man für die drei Serien jeder Sorte die zwischenliegende Serie als Standard wählt, erhält man die Werte der Tab. 3.

TABELLE 3. *Entfernung der Mittelwerte jeder Sorte in den drei Seriengruppen von dem Mittelwert der zwischenliegenden Serie, in deren Standardabweichung ausgedrückt.*

Sorte	Signum	Seriengruppe I	Seriengruppe II	Seriengruppe III
Derenburg 8	2	$0 \pm 0{,}039$	$-0{,}03 \pm 0{,}042$	$+0{,}12 \pm 0{,}039$
Janetzki Original	3	$-0{,}07 \pm 0{,}049$	$+0{,}47 \pm 0{,}045$	$0 \pm 0{,}039$
Mansholt, Groningen, Original.....	4	$0 \pm 0{,}043$	$+0{,}10 \pm 0{,}050$	$-0{,}59 \pm 0{,}042$
Eckendorfer Original	5	$-0{,}21 \pm 0{,}041$	$+0{,}10 \pm 0{,}051$	$0 \pm 0{,}040$

In den Tabellen sind also beträchtliche Unterschiede beobachtet worden, und zwar sowohl zwischen verschiedenen Sorten als auch innerhalb derselben Sorte in verschiedenen Bestimmungsserien. Letztere Tatsache zeigt, wie oben angeführt, dass im Materiale nicht bloss Milieumomente (oder Unterschiede der Gradierungstechnik) vorhanden sind, die sich zufälligerweise bei den verschiedenen Pflanzen der Bestimmungsserien geltend machen, sondern dass sich auch systematische Momente geltend machen. Damit meinen wir solche, die nicht nur eine einzige, sondern mehrere benachbarte Pflanzen beeinflussen. Es ist selbstverständlich, dass das Vorkommen eines oder einiger weniger zufällig zusammentreffender, systematischer Milieumomente leicht einer ganzen Parzelle eine bestimmte Prägung aufzudrücken vermag.

Können dann Verhältnisse dieser Art die in den Tabellen ersichtlichen Unterschiede zwischen den Sorten verursacht haben? Bevor

dieses Problem klargestellt worden ist, besteht natürlich keine Gewähr dafür, dass die Tabellen tatsächlich vorhandene Unterschiede zwischen den verschiedenen Sorten widerspiegeln.

Früher haben wir die verschiedenen Pflanzen als Untersuchungseinheiten betrachtet. Wir wollen nun die Parzellen als Einheiten anwenden, was jetzt dank der in Tab. 2 angeführten Mittelwerte möglich ist. Erst wenn wir dies tun, können wir sicher sein, die Schlusssätze von den genannten Faktoren unabhängig zu machen.

Man kann da infolge der Ordnung der Parzellen auf dem Versuchsfelde von den systematischen Milieumomenten absehen und die Abweichungen der Werte der Parzellen, insoweit sie milieubedingt sind, als durch den Zufall bedingt auffassen.

Dass innerhalb der Seriengruppen grössere Unterschiede der Messtechnik vorhanden sein sollten, dürfte ausgeschlossen sein, und dass die Bestimmungstechnik von Seriengruppe zu Seriengruppe in höherem Masse verschiedenartig sein sollte, ist (vgl. S. 196) wenig wahrscheinlich (und die erhaltenen Resultate deuten auch nicht darauf hin). Daher dürfte man vielleicht von der Möglichkeit absehen können, dass systematische Unterschiede der Bestimmungstechnik zwischen den verschiedenen Parzellen vorliegen, und die Werte der Sorten (Tab. 2) unabhängig von den Seriengruppen vergleichen. Wir wollen jedoch nicht ganz von der Möglichkeit systematischer, bestimmungstechnischer Unterschiede zwischen den Seriengruppen absehen und führen daher parallel zu einem Vergleiche unabhängig von den Seriengruppen (Alternative II) einen Vergleich zwischen den Differenzen der Sorten innerhalb jeder Seriengruppe (Alternative I) durch.

Da die Parzellen somit als Untersuchungseinheiten betrachtet werden und also in den beiden Alternativen nur ein sehr kleines Material zur Verfügung steht, lassen uns die üblichen statistischen Methoden im Stiche. Es gibt jedoch Methoden, die für kleines Material [vergl. PEARSON (1915)] zugeschnitten sind und die hier angewendet werden können, für Alternative I eine Methode, die von »STUDENT» (1908) aufgestellt worden ist, für Alternative II eine von FISHER (1925, vergl. 1930) gegebene Methode. Beide bedingen in vorliegendem Fall bei einem Vergleiche zwischen zwei Sorten, dass auf die Differenz zwischen den Mittelwerten ihrer Parzellenwerte der bekannte von »STUDENT» (1908, vergl. 1917) aufgestellte und später [FISHER (1915, 1925), PEARSON (1915)] mathematisch verifizierte Satz angewendet wird, nämlich dass das Integral

$$p = 0{,}5 + \frac{1}{\sqrt{\pi}} \cdot \frac{\Gamma\left(\frac{n}{2}\right)}{\Gamma\left(\frac{n-1}{2}\right)} \int_0^z (1+z^2)^{-\frac{n}{2}} \, dz$$

die Wahrscheinlichkeit dafür ergibt, dass ein Mittelwert einer Gruppe von n Einheiten, die zufällig einem (unendlich grossen) normal verteilten Material entnommen sind, nicht z übersteigen werde, wobei z die Entfernung vom Mittelwerte des Gesamtmateriales bezeichnet, der in der Standardabweichung der *Gruppe* ausgedrückt ist. (Anstatt z führt FISHER $t = z\sqrt{n-1}$ ein). Betreffs der Prinzipien dieser nun immer mehr bekannten Methoden sei auf Arbeiten der obengenannten Forscher und anderer (hauptsächlich in Biometrika und Metron) hingewiesen [1].

Die Resultate bei der Anwendung dieser Methodik auf die Ziffern in Tab. 2 wurden in Tab. 4 zusammengestellt. Gewisse Werte der Alternative I, woselbst z zu hoch war, um in den vorhandenen Tabellen für p (siehe Fussnote) aufgefunden werden zu können, sind direkt mit Hilfe des »STUDENT»schen Integrals für $n = 3$ [in der für praktische Rechenarbeit geeigneten Form $p = 0{,}5\ (1 + \sin \Theta)$, wobei $\Theta = \text{arctang } z$] berechnet worden.

Wir vergleichen zuerst Janetzki Original (3) und Derenburg 8 (2). Wenn in der Rostempfänglichkeit kein tatsächlicher Unterschied zwischen diesen beiden Sorten vorläge und die in Tab. 2 zutage getretenen Differenzen zwischen den betreffenden Parzellenwerten jeder einzelnen Seriengruppe (Alternative I) als durch milieubedingte und bestimmungstechnische Verhältnisse verursachte, zufällige Abweichungen von einem aprioristischen Mittelwerte 0 eines normal verteilten Materiales anzusehen wären, so könnte man etwa 262 gegen 263 (= 9962 gegen 10000) setzen, dass sich zufällig eine niedrigere mittlere Differenz als die beobachtete $2{,}_{08}$ ergeben hätte. Diese Wahrscheinlichkeit entspricht in der Normalkurve einem Unterschied zwischen Derenburg 8 und Janetzki Original vom $2{,}_{7}$-fachen des mittleren Fehlers.

Sicherlich dürfte man jedoch den Vergleich unabhängig von den Seriengruppen ausführen können (Alternative II). Dabei erhält man als entsprechende Wahrscheinlichkeit ungefähr 8849 gegen 8850, also (wie hier ohne weiteres zu erwarten war, da die Parzellenwerte, die

[1] Angaben, wie die Rechenarbeit praktisch auszuführen ist, sowie Tabellen für p findet man in FISHER (1930), PEARSON (1914), »STUDENT» (1908, 1917, 1925) und TEDIN (1926).

derselben Seriengruppe angehören, negativ korreliert sind) einen höheren Wert, der in der Normalkurve einem Unterschied vom 3,8-fachen des mittleren Fehlers entspricht.

Für die beiden dazwischenliegenden, einander näherstehenden Sorten (s. Tab. 4) Mansholt, Groningen, Original (4) und Eckendorfer Original (5) ergeben sich beim Vergleiche mit Janetzki Original (3) und Derenburg 8 (2) hohe Wahrscheinlichkeiten. In der Normalkurve entsprechen diese für Mansholt, Groningen, Original in Alternative I etwa dem 2-fachen, in Alternative II ungefähr dem 2,5-fachen des mittleren Fehlers; für Eckendorfer Original in Alternative I etwa

TABELLE 4. Wahrscheinlichkeit (*p*) *für das Zurückbleiben (in algebraischem Sinne) einer Abweichung zweier Sorten hinter der beobachteten (positiven) Abweichung und Multiplum (α) des mittleren Fehlers, das in der Normalkurve der Wahrscheinlichkeit p entspricht.*

Verglichene Sorten[1]. (Die von Rost am meisten angegriffene Sorte wird zuerst genannt.)	Differenz zwischen dem Mittelwert der Parzellenwerte der ersten Sorte und dem der zweiten	Verhältnisse des Milieus und der Messtechnik werden als zwischen			
		den Parzellen jeder Seriengruppe		sämtlichen Parzellen	
		zufällig wirkend vorausgesetzt			
		I. »STUDENT«S Methode		II. FISHERS Methode	
		p	*α*	*p*	*α*
3 und 2.....	2,08	0,9962	2,7	0,9999	3,7
3 und 4.....	1,15	0,9871	2,2	0,9945	2,5
3 und 5.....	1,21	0,9974	2,8	0,9989	3,1
4 und 2.....	0,93	0,9663	1,8	0,9936	2,5
5 und 2.....	0,88	0,9928	2,4	0,9995	3,3
4 und 5.....	0,06	0,5831	0,2	0,5908	0,2

[1] Bedeutung der Ziffern: 2 = Derenburg 8; 3 = Janetzki Original; 4 = Mansholt, Groningen, Original; 5 = Eckendorfer Original.

dem 2,5-fachen, in Alternative II ungefähr dem 3-fachen des mittleren Fehlers.

Den obigen Resultaten muss ein Vorbehalt noch beigefügt werden. Da die Klasseneinteilung bei vorliegender Untersuchung ziemlich grob ist, dürfte es angebracht sein, kurz zu erörtern, inwiefern die erhaltenen Resultate durch Unterschiede zwischen den Klassenmittel-

werten der Standardserie und denjenigen der untersuchten Serien beeinflusst sein können. Schon beim Durchsehen von Tab. 1 b dürfte es einleuchten, dass betreffs der geschlossenen Klassen und der obersten offenen Klasse Unterschiede, welche die Resultate in höherem Ausmasse verschieben können, kaum anzunehmen sind. Ich habe jedoch für die geschlossenen Klassen (1—2, 2—3, 3—4) die Lage der Klassenmittelwerte der verschiedenen Serien zwischen den Klassengrenzen unter Voraussetzung normaler Verteilung bestimmt und einen Vergleich mit der Lage der Klassenmittelwerte der Standardserie durchgeführt. Dabei wurde konstatiert, dass, wenn man, anstatt an dem gesamten Material die aus der Standardserie berechneten Klassenmittelwerte für die inneren Klassen anzuwenden, zwischen die Klassengrenzenwerte der Standardserie (unter Voraussetzung normaler Verteilung in den untersuchten Serien) interpollierte Klassenmittelwerte anwendete, eine Verschiebung der in Tab. 2 mitgeteilten Mittelwerte sich bloss unbedeutend in der zweiten Dezimalstelle geltend machen würde.

Für die unterste Aussenklasse (0—1) liegt jedoch die Möglichkeit vor, dass zwischen den verschiedenen Serien in Tab. 1 b Verschiebungen der Klassenmittelwerte vorhanden sind, welche in beträchtlichem Ausmasse die Vergleiche stören können. Besonders naheliegend ist es, die Möglichkeit zu erwägen, ob der Klassenmittelwert 0—1 für Derenburg 8, von welcher Sorte die betreffende Gruppe nicht weniger als etwa 70 % der Pflanzen enthält, so erheblich von dem Klassenmittelwerte der Standardserie abweichen könne, dass die Schlussätze gefährdet werden. Auf Grund der gleichartigen Verteilung der drei Bestimmungsserien von Derenburg 8 dürfte man jedoch davon ausgehen können, dass die Klassenmittelwerte 0—1 dieser Sorte in diesen drei Bestimmungsserien nicht wesentlich voneinander abweichen.

Natürlich hat man keine Möglichkeit, exakt zu entscheiden, wo der Klassenmittelwert 0—1 für Derenburg 8 liegt. Es wäre unberechtigt, hier ohne weiteres normale Verteilung vorauszusetzen. Nimmt man an, der Klassenmittelwert liege bei der Klassengrenze 1, so hat man mit einem zu hohen Werte gerechnet. Der Wert der Abszisse an der Klassengrenze 1 beträgt — 1,02, wobei wie oben der Mittelwert dieser Serie als 0-Punkt und deren Standardabweichung als Masseinheit gewählt worden ist. Setzt man den genannten Wert für Derenburg 8 in die Formel (6) als Klassenmittelwert der Gruppe 0—1 ein anstatt des früher aus Formel (5) berechneten Wertes — 1,54 (die übrigen Klassenmittelwerte werden beibehalten), so erhält man für die betreffende Sorte in

den Seriengruppen I, II bezw. III die Maximalmittelwerte — 0,75, — 0,81 bezw. — 0,71.

Es ergibt sich also eine neue Serie von Parzellenwerten, deren Mittelwert — 0,76 einen sicheren Maximalwert darstellt und dem der übrigen Sorten beträchtlich näher steht, deren Standardabweichung aber geringer als diejenige der früher in Tab. 2 für Derenburg 8 erhaltenen Parzellenserie ist.

Wenn man beim Vergleich zwischen Derenburg 8 und den übrigen Sorten unter Anwendung dieses letzteren Mittelwertes, aber der früheren Standardabweichung von Derenburg 8 FISHERS Methode benutzt, so besteht gute Gewähr dafür, dass die Werte von z und damit auch die von p, welche man erhält, Minimalwerte sind. (Die Methode besagt ganz einfach, dass man anstatt des früher angewendeten z — bezw. t nach FISHER — den Wert $\frac{z\ (M_B - \overline{M}_A)}{M_B - M_A}$ anwendet, in welchem M_A der früher, $\overline{M}_A$ der später erhaltene Parzellenmittelwert für Derenburg 8 ist; M_B ist der Parzellenmittelwert derjenigen Sorte, mit welcher der Vergleich ausgeführt wird).

Auch nun erhält man hohe Werte von p. Beim Vergleich zwischen Derenburg 8 (2) einerseits und Janetzki Original (3), Mansholt, Groningen, Original (4) bezw. Eckendorfer Original (5) andererseits ergeben sich die p-Werte 0,9998, 0,9673, bezw. 0,9963, die in der Normalkurve dem 3,5-, 1,8- bezw. 2,7-fachen des mittleren Fehlers entsprechen.

Aus Tab. 1 und 2 findet man, dass Derenburg 8 in den untersuchten Parzellen beträchtlich weniger als Janetzki Original vom Gelbroste angegriffen ist sowie dass die beiden Sorten Mansholt, Groningen, Original und Eckendorfer Original eine Mittelstellung zwischen den beiden ersten Sorten einnehmen. Auf Grund der Resultate in Tab. 4 kann man nun (auch wenn betont werden muss, dass die Werte von p wiederum natürlich zufallsbedingt sind) schliessen, dass diese Tatsachen schlechthin kaum auf milieu- und messtechnische Verhältnisse zurückgeführt werden können, sondern auf wirklichen Unterschieden in der Rostempfänglichkeit der untersuchten Sorten beruhen müssen.

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DAS VORKOMMEN VON LANDSTEINER's "IMMUNRECEPTOREN" M UND N IN DER DÄNISCHEN BEVÖLKERUNG

VON *OLUF THOMSEN* UND *JOHS. CLAUSEN*
UNIVERSITÄTSINSTITUT FÜR ALLGEMEINE PATHOLOGIE, KOPENHAGEN

BEKANNTLICH haben LANDSTEINER und LEVINE (1927; 1928 a, b; 1929 a, b) bei ihrer Arbeit mit der Herstellung gruppenspezifischer Immunsera (Kaninchen) bisher unbekannte, M, N und P [1] benannte Blutkörperchenreceptoren nachgewiesen. Für diese Receptoren ist Agglutinin im Serum von Menschen nicht enthalten, u. zw. auch nicht von solchen, deren Erythrocyten die entsprechenden Receptoren fehlen. Es ist doch fraglich, ob dieser Satz stichhaltig ist. Die Agglutinationsreaktion ist ein verhältnismässig grober Indikator für das Vorhandensein eines Antikörpers und das eigentliche Agglutinationsphänomen hängt sowohl von der absoluten Stärke des Agglutinins als auch von der quantitativen und qualitativen Entwicklung (»Empfindlichkeit») des Receptors ab. An und für sich ist es nicht unwahrscheinlich, dass Menschensera wirklich Anti-M bzw. Anti-N enthalten, immerhin aber unter demjenigen Schwellenwert, bei welchem Agglutination, und damit eben Nachweisbarkeit, eintritt. Es wäre möglich, dass ein feinfühligerer Indikator, als die Agglutination es ist, das Vorhandensein homologen Antistoffs ebenso wie die übrigen Isoantikörper »reziprok» aufdecken würde.

Vorderhand lassen die Receptoren sich indessen nur mit Hilfe tadelloser gereinigter Immunsera [2] nachweisen. Diesen Nachweis haben wir teils durch Mischung eines Tropfens Immunserum (bzw. verschiedener Verdünnungen davon) mit einem Tropfen 1—2 % Blutsuspension auf einem Objektträger, teils durch die übliche Austitrierung in einer Reihe Reagensgläser (0,1 ccm verdünntes Serum + 0,1 ccm Blutsuspension) ausgeführt. Ablesung sowohl makro- als auch mikro-

[1] Da P in der europäischen Bevölkerung nur ausnahmsweise angetroffen wird, ist dieser Receptor nicht berücksichtigt worden.

[2] Zur Identifizierung unserer ersten Blutproben als M+ und N+ benutzten wir Immunsera, die uns von Dr. LANDSTEINER (New York) und von Dr. SCHIFF (Berlin) gütig zur Verfügung gestellt wurden. Für diese freundliche Beihilfe sprechen wir den beiden Herren unseren verbindlichsten Dank aus.

skopisch nach 1—2 Stunden. Insgesamt wurden 580 isolierte Proben untersucht (442 »gesunde» Individuen und 138 Patienten mit verschiedenen Krankheiten). Die Verteilung zwischen Hauptgruppen (0, A, B, AB) und »Untergruppen» (M, MN, N) ist in den Tabellen 1 und 2 wiedergegeben.

TABELLE 1. *Verteilung der Hauptgruppen (0, A, B, AB) in den 3 Untergruppen (M, MN, N) bei 442 gesunden Individuen (Kopenhagen).*

		0	*A*	*B*	*AB*	*Insgesamt*
M	♂	17	26	10	5	58
	♀	13	13	4	2	32
	×	17	16	8	1	42
	Insgesamt	47 (35,6 %)	55 (41,7 %)	22 (16,7 %)	8 (6 %)	132 (100 %)
MN	♂	29	28	7	1	65
	♀	17	36	8	5	66
	×	26	32	5	3	66
	Insgesamt	72 (36,6 %)	96 (48,7 %)	20 (10,2 %)	9 (4,6 %)	197 (100 %)
N	♂	18	24	8	0	50
	♀	10	19	4	2	35
	×	14	9	2	3	28
	Insgesamt	42 (37,2 %)	52 (46 %)	14 (12,4 %)	5 (4,4 %)	113 (100 %)
	Summe	161 (36,4 %)	203 (46 %)	56 (12,6 %)	22 (5 %)	442 (100 %)

× = Geschlecht nicht angegeben.

TABELLE 2. *Die prozentische Verteilung von M, MN und N in den Hauptgruppen 0, A, B, AB ist folgende:*

	M	MN	N
Gruppe 0	29,2	44,7	26,1
» A	27,1	47,3	25,6
» B	39,3	35,7	25,0
» AB	36,4	40,9	22,7
Insgesamt	30	44,6	25,5

Die Häufigkeit von M (in den Untergruppen M und MN) beträgt 74,6 %
» » » N (in den Untergruppen N und MN) » 70,1 %
» » » MN » 44,6 %

Innerhalb der Fehlergrenzen wird demnach die nämliche Verteilung der Hauptgruppen in den Untergruppen und der Untergruppen in den Hauptgruppen angetroffen, und das zeigt, dass zwischen Haupt- und Untergruppen keine Korrelation besteht, oder m. a. W., dass sie ver-

schiedenen, voneinander unabhängigen Systemen angehören. Das entspricht völlig den von LANDSTEINER und LEVINE und von SCHIFF (1929, 1930) erzielten Resultaten.

Es wurden ferner 138 an verschiedenen Krankheiten leidende Patienten untersucht. Diese wurden doch nur auf M (M+) oder Fehlen von M (M—) untersucht. Sie weisen die nämliche Verteilung von + und — auf.

	0	*A*	*B*	*AB*	Insgesamt
M+	56	32	5	6	104 (75,5 %)
M—	17	11	3	2	34 (24,5 %)
	73	43	8	8	138 (100 %)

Schliesslich wurden die Erblichkeitsverhältnisse bei 105 zum grössten Teil neugeborenen Kindern und deren Eltern untersucht:

Elternkombinationen	*M*	*MN*	*N*
M × M	13	—	—
M × N	—	14	—
N × N	—	—	5
M × MN	12	17	—
N × MN	—	11	9
MN × MN	8	9	7

LANDSTEINER und LEVINE haben verschiedene Möglichkeiten für die erbliche Grundlage erörtert, und da das gleichzeitige Fehlen von M und N in ihrem Material nicht vorgekommen ist, sind sie eher geneigt zu glauben, dass die beiden dominierenden Eigenschaften, die Entwicklung der Receptoren M und N, auf Grundlage zweier allelen Gene (M und N) entstehen. Auch SCHIFF hält diese Hypothese auf Grundlage eines beträchtlichen Materials und bevölkerungsstatistischer Berechnungen für richtig. Hiernach müssten M-Individuen ebenso wie N-Individuen stets homozygot sein, während MN-Individuen heterozygot sein müssten. Gleichwie die genannten Forscher haben wir die Beobachtung gemacht, dass die Agglutination bei reinen M- und N-Individuen regelmässig intensiver ist (grösser Flocken oder Klumpen) als bei MN-Individuen, was mit der Annahme, die ersteren seien homozygot, ja trefflich in Einklang steht.

Nur SCHOCKAERT (1929; Belgien) hat gleichzeitiges Fehlen von M und N, u.zw. in nicht weniger als 11 Proz. der Untersuchten, festgestellt. Da die Prozentzahlen für +M und +N in dieser Untersuchungsreihe aber erheblich niedriger sind als die in Amerika (LANDSTEINER) sowie in Berlin (SCHIFF) und Kopenhagen (THOMSEN und CLAUSEN) ermit-

telten, so kann man höchst wahrscheinlich von der Voraussetzung ausgehen, dass technische Mängel daran schuld sind, dass das Prozent von »Positiven» zu niedrig ist. Es wäre doch ganz unwahrscheinlich, dass die Bevölkerung Belgiens sich in bezug auf M und N wesentlich anders verhielte als die Bevölkerungen anderer Länder.

Wie man sieht, enthält unser — allerdings nicht sehr grosses — Material keine Abweichungen von dem, was man als Resultat von zwei allelen Genen, von denen keines in dem Sinne dominiert, dass das eine die Wirkung des anderen unterdrückt, erwarten müsste. Wir müssten alsdann in der MN-Gruppe eine Analogie zur AB-Gruppe im Hauptsystem haben. SCHIFF hat in seinem verhältnismässig beträchtlichen Erblichkeitsmaterial auch keine Abweichungen, wohingegen LANDSTEINER und LEVINE (1928 b) in 64 Ehen mit 286 Kindern fünf »Abweichungen» verzeichnen. Diese müssen also — wenn das System für richtig gelten darf — entweder als Folge von »Illegitimität» (falscher Vater) oder auf dem Versagen der Technik beruhend betrachtet werden, was sich schwerlich ganz von der Hand weisen lässt, da sie aus einer Zeit stammen, wo man in bezug auf die verlässlichste Methodik noch nicht so viel Erfahrung besass wie heute. Es verdient doch Beachtung, dass es bei den erwähnten fünf »Abweichungen» der Vater ist, der Schwierigkeiten bereitet, und dieser Umstand kann natürlich für Illegitimität sprechen. Andererseits sind fünf »illegitime Fälle» auf 286 natürlich recht reichlich, da man nicht vergessen darf, dass »Illegitimität» sich keineswegs in der Gruppe des Kindes zu erkennen zu geben braucht. Ein grösseres Material, das allmählich gesammelt werden kann, dürfte darüber endgültigen Aufschluss und Sicherheit bringen. Die nachstehende Verteilung von M und N müsste ja in den verschiedenen möglichen Elternkombinationen angetroffen werden:

	Nachkommenschaft			
Elterkombination	Proz.	M	MN	N
M × M		100	—	—
M × N		—	100	—
N × N		—	—	100
M × MN		50	50	—
N × MN		—	50	50
MN × MN		25	50	25

Unsere eigenen Zahlen stehen hiermit, in Anbetracht der verhältnismässig niedrigen Anzahl, so gut in Einklang, wie man von vorn herein erwarten konnte. In bezug auf die Technik sei folgendes bemerkt:

Die Herstellung kräftig wirksamer Anti-M-Immunsera gelingt durchgehends leichter als die Herstellung entsprechender Anti-N-Sera.

Auch wir haben das von LANDSTEINER und LEVINE geschilderte eigentümliche Verhalten wahrgenommen, dass bei der Absorption mit Blut ohne N zur Reinigung von Anti-N-Sera ein so erheblicher Teil von Anti-N mit entfernt wird, dass dieses durch wiederholte Absorptionen gänzlich entfernt werden kann und das Serum zuletzt unwirksam wird. Dies könnte möglicherweise dafür sprechen, dass das Blut aller Menschen N-Substanz enthält, das Blut »N-negativer» aber in so winziger Menge, dass es durch seine Absorptionsfähigkeit zwar nachweisbar wird, aber nicht direkt durch Agglutination.

Möglicherweise trifft das auch — obwohl in geringerem Masse — für den M-Receptor zu. Die Rolle des M- und des N-Gens müsste alsdann die sein, die Entwicklung des homologen Receptors über die »latente» Entwicklung emporzuheben.

In allen den Fällen, wo bei unseren Bestimmungen der Gruppe (M, MN, N) auch nur der leiseste Zweifel herrschte, haben wir die direkte Agglutinationsprobe mit der spezifisch absorbierenden Fähigkeit des Blutes kombiniert, denn es dürfte doch keinem Zweifel unterliegen, dass jedenfalls in quantitativer Hinsicht für M wie auch für N ein ausgesprochener Unterschied zwischen + und — besteht.

Ebenso wie LANDSTEINER und LEVINE haben wir ermittelt, dass die Reinigung der Anti-N-Sera bei 37° C leichter gelingt als bei Zimmertemperatur oder noch niedrigerer Temperatur.

ZUSAMMENFASSUNG.

Bei der Untersuchung von 442 »gesunden» Individuen (Kopenhagen) und 138 Patienten mit verschiedenen Krankheiten wurde M+ bei 74,6 Proz. und N + bei 70,1 Proz. ermittelt. Sowohl M als auch N wurde bei 44,6 Proz. angetroffen. Gleichzeitiges Fehlen von M und N wurde in keinem einzigen Falle festgestellt.

Bei der Untersuchung von 105 grösstenteils neugeborenen Kindern und deren Eltern wurden, LANDSTEINER und LEVINE's Angaben gemäss, M und N als voneinander unabhängige, dominierende Eigenschaften ermittelt. Die Untersuchungen bestätigen die Annahme, dass die drei möglichen Gruppen M, MN, N auf der Kombination von zwei allelen oder alternierenden Genen beruhen. Diese müssen in einem anderen Chromosomenpaar enthalten sein als die Gene der Hauptgruppe, und die drei Gruppen M, MN und N treten nun als »Untergruppen» auf, die

auf die vier (0, A, B, AB) bzw. sechs Hauptgruppen (0, A^1, A^2, B, A^1B, A^2B) gleichmässig verteilt sind. Durch die Kombination der beiden Systeme lässt die Menschheit sich somit in 6×3 »Gruppen» einteilen, woraus sich eine erheblich vermehrte Möglichkeit (bis etwa 40 Proz.) ergibt, »unrichtige Vaterschaft» in gerichtsmedizinischen Paternitätssachen auszuschliessen.

Die Untersuchungen sind nun so weit gediehen und die Methodik so verlässlich, dass die vollständige Differenzierung der Entscheidung von Vaterschaftssachen nunmehr als festes Glied angereiht werden muss. Selbst wenn man Abweichungen von dem »Untergruppensystem» noch keine absolute Bedeutung beimessen will, so muss man sie doch als gewichtige Indizien benutzen können.

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CYTO-GENETIC AND TAXONOMIC INVESTIGATIONS ON MELANIUM VIOLETS

BY *J. CLAUSEN*
GENETICS LABORATORY OF THE ROYAL VETERINARY AND AGRICULTURAL COLLEGE, COPENHAGEN

I. INTRODUCTION.

THE *Melanium* section of the genus *Viola* offers exceptional opportunities for a comparative study of hybrids between species with different numbers of chromosomes as well as for experimental taxonomic studies on the relationship and the origin of species. These opportunities are due to the facts that a unique series of numbers of chromosomes ranging from n = 7 to n = abt. 30 occurs in this section and that each species is capable for crossing with a number of other species, irrespective of their chromosome numbers, giving at least partially fertile hybrids. This makes possible a cyto-genetic analysis of the differences characterising the individual species of an entire section, or rather sub-genus, thus interlinked by fertile specific hybrids. The chief intention for the present study is, therefore, to clear up how the species of one natural sub-genus are constructed, cyto-genetically speaking, or at least to throw new light upon this problem.

The genetical part of the analysis, thus defined, was initiated with KRISTOFFERSON's investigations on hybrids between *Viola arvensis* and *tricolor* (KRISTOFFERSON 1914, 1916, 1923); in the last named paper a few notes on hybrids of *Viola Munbyana* with *tricolor* and *arvensis* are added. The present author contributed with a series of papers on the taxonomic and cyto-genetic analysis of *Viola tricolor* and *arvensis* (J. CLAUSEN 1921, 1922, 1926, 1927 a and 1930 a) and on cytology and taxonomy of a number of *Viola* species (1927 b, 1929, 1930 b and 1931 b).

Some of the experiments published here date back as far as to 1922 and 1924, but the main part of them have been under way only the last five years, from 1926. A series of species were selected for this comparative investigation. This selection was partly a natural one, because most alpine species and some of the subalpine ones do not succeed under the conditions of a lowland experiment field, and some species as for instance *lutea* and *Battandierii* suffer also unquestionably from inbreeding. In such cases propagation by selffertilisation was

impossible, and it was found necessary to take recourse to individuals produced by uncontrolled pollination in the open.

Fourteen species representing the haploid chromosome numbers 7, 10, 11, 13, 17, 18, 20, 24 and abt. 26—30 (oscillating) enter in the crossings. 38 different specific crossings were effected; of these the 23 were successful, inasmuch as they gave flowering F_1-plants. Probably repeated crossing using other varieties would in some cases have given a better result. Thus, for instance, *V. Battandierii* crossed with the *alba*-yellow variety of *tricolor* gave in two reciprocal directions 24 plus 14 seeds, but only one plant came to flowering in addition to a dwarfish and very cespitose one, which for three on each other following years did not flower at all. On the other hand, the same specific cross, but applying the *hortensis* variety (Line 519, $n = 13$) as the *tricolor* parent, yielded a number of vigorous, abundantly flowering and comparatively fertile F_1-plants. A similar difference was seen among the hybrids of *V. lutea* with *tricolor*: using the var. *hortensis* of *tricolor* as one parent, a vigorous hybrid was obtained, while the hybrid of *V. lutea* with *tricolor alba* was very weak and suffered from »black leg», just as the inbred *V. lutea* itself.

Only some of the crossings were reciprocal. The annual species were often a little late for the perennial ones, so that these last ones had to be used as male parents or, if both were sown the same year, as females alone. In one case, namely *V. arvensis* × *rothomagensis*, the crossing was possible only in one direction; the cross with *arvensis* as the female parent gave many good and well germinating seeds, while the reciprocal cross with *rothomagensis* as mother in eight repeated instances invariably gave weak and shrunken seeds, which did not germinate at all. Both of these species have the haploid number of 17 chromosomes. The different success of the reciprocal crossings is therefore not connected with any difference as to the number of chromosomes in the endosperm of the reciprocal crosses, as THOMPSON (1930 a, b) showed for wheat crosses. Two plausible explanations are here suggested, namely either disturbed genic equilibrium caused by two sets of *rothomagensis*-chromosomes with one set of *arvensis*-chromosomes being brought together in the hybrid endosperm, or incompatibility between the F_1 chromosomic complement and the plasm of *rothomagensis*. In the eight other cases, where reciprocal hybrids were obtained, no differences between the two reciprocals were observed. If such were present, they would be so slight as to be uncertain at all.

The technique was mainly the same as described in the 1926-paper:

emasculation with a needle, pollination 4—6 days later and the capsules bagged in parchment bags some time before ripening in order to prevent the seeds from being spread. Sowing was effected in March in hot beds and the plants planted out in the field in May. Plants for self-fertilisation were removed to the insect proof green house, and at least all large flowered ones had to be artificially pollinated with a needle. Some of the very sterile hybrids were back-crossed. This gave more seeds. For fixation CARNOY's fluid was applied during the first years; 1927 NAWASHIN's chromic-acetic acid was tried for some of the fixations, and the last three years the combination of immersion for 5—10 minutes in CARNOY's fluid followed by abt. 24 hours in NAWASHIN's fixative proved very successful. The slides made before 1927 were stained mainly with HEIDENHAIN's iron-alum hæmatoxylin, the more recent ones with iodine-gentian violet.

The research work was done almost exclusively during leisure hours, and it had not been possible to get through without the kind and very accurate assistance yielded by my wife, Fru ANNA CLAUSEN, during the seasonal work on the experiment field. Artificial pollinations, back-crossings, fixations, baggings and harvesting were made almost exclusively by her, and she assisted me also in the enumeration of segregated types. Through the years the investigations were financially supported by funds from the Carlsberg Foundation, which deliberated me from taking paid work during the time left from departmental work, enabling me to devote such time to research. Part of the cytological investigations were done during a research fellowship of the International Education Board from September 1927 to May 1928, spent at Division of Genetics, University of California, Berkeley, in my friend, Professor E. B. BABCOCK's laboratory. The remaining part of the work was done at the Genetics Department of the Royal Veterinary and Agricultural College, Copenhagen, in close and friendly connection with Professor Dr. ÖJVIND WINGE. Grateful acknowledgements are here extended to the institutions and single persons, who in one or another way, also by supplying of seeds of wild species, have supported these investigations.

The scope of this paper will be to extract from the records of the experiments such data, which might be supposed to contribute to the clearing up of problems of a more general biological interest, and to select some few crossings for a more detailed report; these may then be taken as paradigms for specific hybridisations in the *Melanium* section of *Viola*.

II. SPECIFIC MATERIAL, WITH A SURVEY ON HYBRIDISATIONS.

The diagram, fig. 1, reports the species applied and the hybridisations made. The species are arranged after increasing chromosome number with one exception, namely those belonging to the collective species *V. Kitaibeliana*, which are inserted in one group after *arvensis*. Successful hybridisations (such that gave flowering F_1-plants) are indicated by a full line and a numeral between the two parental

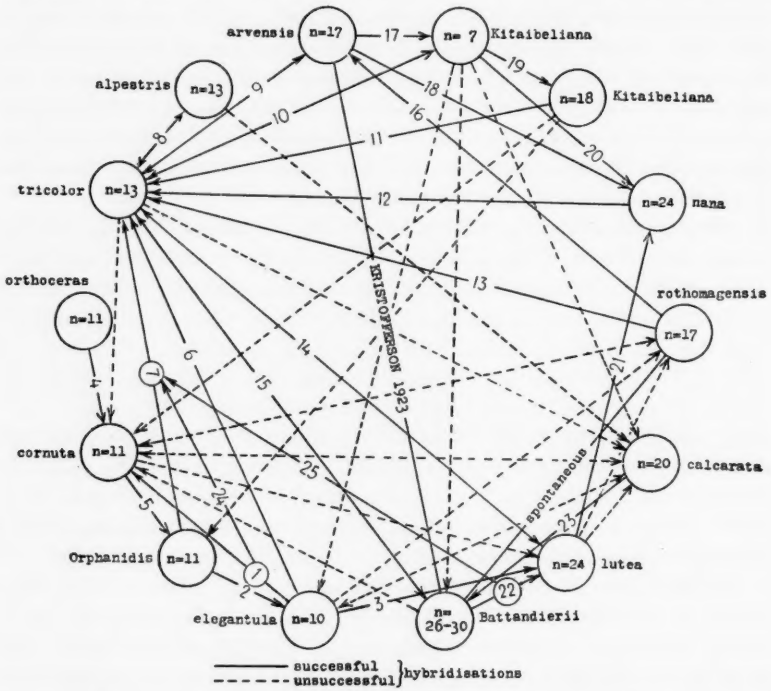


Fig. 1. Diagram of hybridisations in the *Melanium* section. Successful crossings are indicated by a full line between the parental species; the numerals on these lines refer to the cross no. in this paper; the arrows indicate the direction in which the pollen was carried. Stippled lines indicate unsuccessful hybridisations.

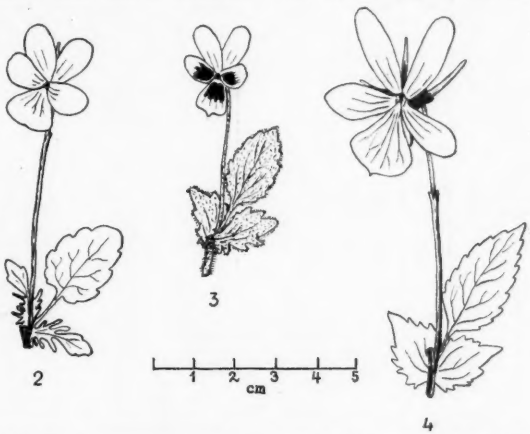
species. The numerals indicate the order in which the hybrids are described in this paper. An arrow indicates the direction in which the pollen was carried. Stippled lines similarly indicate unsuccessful hybridisations. In some cases no seeds were set, in other cases seeds were obtained, but these were not good (shrunken or empty). In most hybridisations, also of the unsuccessful ones, indeed, were good seeds with a plump and apparently good embryo formed, but they would not germinate. A number of them were treated in cold store for a week or two, the testa were caut-

iously removed from some few of them, but nothing would force them to germination. Tables 1 and 2 shortly relate about the hybridisations, the successful and the unsuccessful ones respectively.

In the following, the species applied in the investigations will be introduced to the reader. Many of them are not easily characterised in a brief description, also because they vary very much in nature. For description and figures of chromosomes see J. CLAUSEN 1927 b and 1929. For taxonomic questions compare W. BECKER 1905, 1923 and 1925 a.

Viola elegantula SCHOTT, n = 10.

Syn: *V. latisepala* WETTST., *V. bosniaca* FORMANEK. Illustrations: WITTROCK 1897, Plate VII, figs. 82—88. Distribution: mountains in the northwestern part of Balcan Peninsula (1500—2000 mtrs.). A perennial species, but when cultivated in northern climate generally biennial. Characteristic by its long, straight and very narrow spur, which is thinner than that of *calcarata*. The flowers (fig. 2) are medium-sized, much smaller than *calcarata*'s and almost of the same size as *tricolor*'s. Herbage glabrate, light green, leaves ovate with cordate base, stipules lyrato-pinnatifid. Cultivated in rock gardens, the type applied in the experiments was the commonly cultivated red-flowering one (see the figures in WITTROCK 1897). No spot on the style.



Figs. 2—4. Flowers and leaves of 2: *elegantula*, 3: *Orphanidis* and 4: *cornuta* × *orthoceras* F_1 (Cross 4). All line drawings of flowers and leaves are reduced to abt. $^3/_5$; the scale in centimetres applies to all of them.

Viola Orphanidis BOISS., n = 11.

Syn: *V. prolixa* PANC. Habitat: Balcan Peninsula. Perennial, somewhat prostrate, densely hirsute, stipules somewhat triangular in outline, dentate or a little lobed (fig. 3). Petals rather narrow, violet, often are the three lower ones dark *velutina* nearest to the eye. With or without spot in front of the style. Aberrant chromosomal types with 2n = 21 and 20 chromosomes were previously described (J. CLAUSEN 1930 b). Some of these are pollensterile. The type used for the crossings was the same as described in last named paper.

Viola elegantula and *Orphanidis* no doubt are nearly related and belong in group with a number of Balcan pansies as *V. Nicolai* PANT., *athois* W. BECKR., *dacica* BORB., *Dubyana* BURNAT and *declinata* WALDST. et KIT. Although typical populations of these species exist, the delimitations are in many cases very difficult. For a key to this group, see HAYEK 1917.

TABLE 1. *List of successful hybridisations.* (*I, II, III denote univalents, bivalents etc.*)

Cross No.	maternal species	paternal species	conjugation of chromosomes in F_1	fertility, seeds per plant, F_1	germination	carried to	including backcrosses to	F_1 cultivated year	remarks
1	*cornuta* n = 11	*elegantula* n = 10	ca. $10_{II} + 1_I$ ($1-6_I$), III + IV occur	300—500, not poor	poor, 9 %	F_2	*elegantula*	1925	F_3 did not germinate
2	*elegantula* n = 10	*Orphanidis* n = 10, 11	multivalent association (III, IV, V, VI; I rare)	250—300, rather good	—	F_1	—	1930	spontaneous hybrid
3	*lutea* n = 24	*elegantula* n = 10	$14-15_{II}$, $6-4_I$, autosyndesis, polysomic chains formed	50—100, poor	poor	F_2	—	1928	poor flowering of F_1
4	*cornuta* n = 11	*orthoceras* n = 11	11_{II}, no elimination	rather good	poor	F_2	—	1927	—
5	*Orphanidis* n = 10, 11	*cornuta alba* n = 11	$8-11_{II}$, $8-0_I$, III and IV occur	200—400, rather good	poor, 10—20 %	F_2	—	1927	F_3 weak
6	*tricolor alba*-yellow n = 13	*elegantula* n = 10	$8-10_{II}$, $7-3_I$, polysomes (III—VI) rather common	abt. 30, very poor	—	F_4	*elegantula*, *tricolor*	1927	segregated cespitose, sterile dwarfs
7	*tricolor alba*-yellow n = 13	*Orphanidis* n = 11, 10	$0-11_{II}$, $24-2_I$, some III and IV; I split often	30—130, poor	poor, 24 %	F_4	*tricolor*	1927	—
8	*tricolor* n = 13 *alpestris*	*alpestris* n = 13 *tricolor*	13_{II}, rarely $2-4_I$; extranuclear nucleoli very common	1500—2000, very good	abt. 60 %	F_2	—	1926, 1928	three different reciprocal crosses
9	*tricolor*	*arvensis*	$11-13_{II}$, $8-4_I$	500—2000,	good	F_2	*tricolor*,	1921	11 different

	alpestris	*tricolor*	extranuclear nucleoli very common	very good			—	1928	rent reciprocal crosses
9	*tricolor* n = 13 *arvensis*	*arvensis* n = 17 *tricolor*	11—13_{II}, 8—4_{I}	500—2000, very good	good	F_3	*tricolor*, *arvensis*	1921 and later	11 different reciprocal crosses
10	*tricolor alba* n = 13 *Kitaibeliana* n = 7	*Kitaibeliana* n = 7 *tricolor alba* n = 13	4—6_{II}, 12—8_{I}, occasional III	0—30, very poor	—	F_1	*tricolor*, 10—30 seeds per plant	1930	—
11	*tricolor alba* n = 13	*Kitaibeliana* n = 18	7—12_{II}, 17—7_{I}, non-reduction observed, I split often	20—50, very poor	not good, 23 %	F_2	*tricolor*	1927	—
12	*tricolor alba* n = 13	*nana* n = 24	5—1_{I}, (=16—18_{II}), III and IV occur; autosyndesis	1000—2000, very fertile	—	F_1	*tricolor*	1930	—
13	*tricolor alba*-yellow n = 13	*rothomagensis* n = 17	ca. 1_{IV}, 2—3_{III}, 3—6_{I} (+ bivalents), splitting, elimination	12—37, very poor	poor, 15 %	F_4	*tricolor*	1927	—
14	*tricolor hortensis* n = 13 *lutea*	*lutea* n = 24 *tricolor*	8—11_{I}, mostly splitting; conjugation often in irregular chains	50—300, rather poor	poor, 10 %	F_4	—	1925	*tricolor*, *alba* × *lutea* less viable
15	*tricolor hortensis* n = 13 *Battandierii*	*Battandierii* n = 26—30 *tricolor*	few I; autosyndesis; III + IV	ca. 100, rather good	not good, 25 %	F_2	—	1927	*tricolor alba*-yellow × *lutea* less viable
16	*arvensis* n = 17	*rothomagensis* n = 17	4—6_{I}, often splitting; 1—2_{III}, occasional IV; (+ II)	250—450, rather good	30 %	F_2	—	1926—1928	reciprocal unsuccessful

Cross No.	maternal species	paternal species	conjugation of chromosomes in F_1	fertility, seeds per plant, F_1	germination	carried to	including backcrosses to	F_1 cultivated year	remarks
17	*Kitaibeliana* n = 7	*arvensis* n = 17	most common: $6_{II} + 12_I$; up to $2_{II} + 20_I$; many I split	500—600, rather good, but reduced	poor, 18 %	F_2	—	1926	—
18	*nana* n = 24	*arvensis* n = 17	$2—6_{II}$, $37—29_I$; occasional one III	250—800, rather good	—	F_1	—	1930	—
19	*Kitaibeliana* n = 18 n = 7	*Kitaibeliana* n = 7 n = 18	abt. $6_{II} + 13_I$; sometimes one III	ca. 100, very poor	very poor, ca. 3 %	F_2	—	1929	—
20	*nana* n = 24	*Kitaibeliana* n = 7	$5—6_{II}$, $21—19_I$; III + polysomes occur	250—300, not poor	—	F_1	—	1930	—
21	*nana* n = 24	*lutea* n = 24	$15—18_I$; polysomic conjugation	50—300, rather poor	—	F_1	—	1930	—
22	*lutea* n = 24	*Battandierii* n = 26—30	—	50—100, not good	50 %	F_2	—	1923	—
23	*Battandierii* n = 26—30	*calcarata* n = 20	—	16—60, poor	poor	F_2	—	1929	—
24	Cross 7, F_1 n = 13 × 11, 10	Cross 1, F_1 n = 11 × 10	—	33—325, variable	variable	F_2	—	1928	quadruple hybrid
25	Cross 7, F_1 n = 13 × 11, 10	Cross 1, F_1 n = 24 × 26—30	—	130—150	poor	F_2	—	1928	quadruple hybrid

TABLE 2. *List of unsuccessful hybridisations.*

crossings of	maternal species	paternal species	number of seeds obtained	notes on seeds
elegantula	*elegantula*, n = 10	*Kitaibeliana*, n = 7	8	apparently good
	rothomagensis, n = 17	*elegantula*, n = 10	8	
	elegantula, n = 10	*calcarata*, n = 20	2	empty
	calcarata	*elegantula*	0	
Orphanidis	*Orphanidis*, 2n = 21	*Kitaibeliana*, n = 18	10	
cornuta	*cornuta* violet, n = 11	*tricolor alba*-yellow, n = 13		empty seeds, apparently one good
	cornuta alba, n = 11	*Kitaibeliana* n = 18 (sparse pollen)	0	
	cornuta alba, n = 11 (two types)	*rothomagensis*, n = 17	14	at least four with embryo
			30	
	rothomagensis	*cornuta alba*	2	
	calcarata, n = 20	*cornuta alba*, n = 11	0	
	cornuta alba	*calcarata*	0	
	lutea calaminaria n = abt. 24	*cornuta alba*, n = 11	0	
	cornuta alba, n = 11 (two types)	*Battandierii*, n = 26—30	10	not good
			10	
	Battandierii, n = 26 – 30	*cornuta alba*, n = 11	0	
Kitaibeliana	*Battandierii*, n = 26—30	*Kitaibeliana*, n = 7	7	six of these had good embryos
rothomagensis	*rothomagensis*, n = 17	*lutea*, n = 24	0	
calcarata	*calcarata*, n = 20	*tricolor alba*, n = 13	0	
		tricolor alba-yellow	0	
		alpestris velutina, n = 7	0	
	calcarata, n = 20 (two plants)	*Kitaibeliana*, n = 7	0	at least three out of seven had a good embryo
			15	
	calcarata, n = 20	*lutea*	6	

Viola cornuta L., n = 11.

Illustrations: WITTROCK 1897, plate VII, figs. 93—96. Distribution: High Pyrenees and mountaineous Northern Spain and an isolated locality in Krain (Yugoslavia). Strictly perennial by subterraneous offshoots, very large-flowered with petals very narrow, especially the two upper ones. Spur very long. Petals light violet or pure white, flower fragrant. Two types were applied, one from HAAGE and SCHMIDT, Erfurt, (violet and white flowering plants) and another from H. CORREVON, Geneve (violet flowered); this last one is identical with the wild growing type. Easily cultivated.

Viola orthoceras LEDEB., n = 11.

Transcaucasia 1500—2600 mtrs. Very similar to *cornuta*; mainly characterised from it by larger stipules and shorter petioles. The entire plant is larger than *cornuta*. Very difficult to maintain in culture.

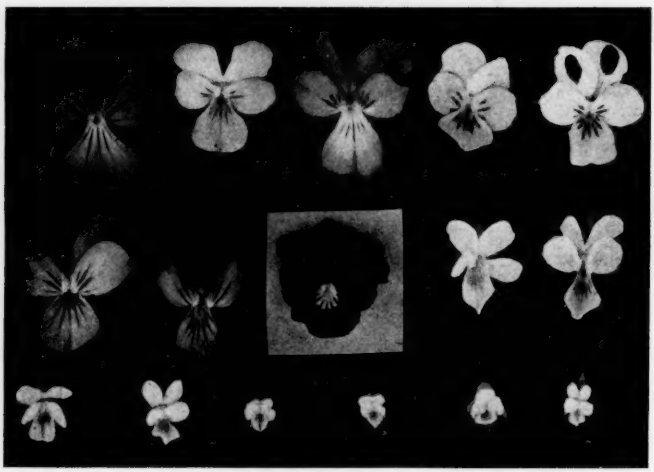


Fig. 5. Flowers of species of the group *Tricolores*; somewhat diminished. — From left, upper row: *Munbyana, lutea calaminaria, rothomagensis, alpestris macedonica* and *alpestris velutina*. — Middle row: *tricolor*-varieties, viz. *typica, maritima rosea* (velvety petals), *nigra, alba* and *alba*-yellow. — Lower row: *arvensis* Line C and Line 52, *Kitaibeliana* (n = 7, n = 8, n = 18) and *nana*.

Both of the species *V. cornuta* and *orthoceras* are exceptional among all other *Melanium* species by having downwards turned lateral petals and by having the centre of the flower (the eye) pure white. All the other species have at least the innermost part of the lower petal intense yellow. The stipules of *cornuta* and *orthoceras* are characteristic broad and triangular; a similar shape of stipules is found only in some species of the *Orphanidis* group, which may be more or less related with *cornuta-orthoceras*. Fig. 4 is from a line drawing of the F_1 hybrid *cornuta* × *orthoceras* and illustrates the morphological characters of leaves and flowers of this group. None of these two species have spot on the style and, notwithstanding their low number of chromosomes, they belong among the largest of the Pansies.

V. tricolor L., n = 13.

For description of variation and types, see WITTROCK 1897 and J. CLAUSEN 1922 and 1926. The typical *V. tricolor* has rather large violet petals and palmate stipules (fig. 5). Most varieties are annuals but subspec. *maritima* is perennial. The varieties used for crossing are: *V. tricolor violacea*, Line 504 (violet flowers, the type of the species), *tricolor alba*, Line 320 (pure white flowers, without anthocyanin), *tricolor hortensis*, Line 519, velvet-violet flowered, an old cultivated garden type, (see J. CLAUSEN 1926, pp. 4—6 regarding these three varieties) and *tricolor alba-lutea* (J. CLAUSEN 1930 a, p. 351), an intense yellow flowering type free from anthocyanin; this type was extracted from the cross *tricolor lutea* × *tricolor alba*. The middle row of fig. 5 shows flowers from a series of *tricolor* varieties.

V. alpestris (DC.) WITTR., W. BECKR., n = 13.

Syn: *V. saxatilis* SCHMIDT (1794). Illustrations: WITTROCK 1897, Plate VI, figs. 77—79. *V. alpestris* is probably only a subalpine subspecies of *tricolor*. Growing on its natural habitats it is perennial; the flowers of most types are bright yellow, sometimes with a *velutina* blotch on the upper petals, those of other types are of a bleached violet colour. The two flowers most to the right in the upper row of fig. 5 belong to *alpestris*; the left one is of the Balcan type (*V. macedonica* BOISS., courteously sent by Professor KOSANIN, Beograd), the right one with *velutina* blotches is from Czechoslovakia (*V. polychroma* KERN.). It is very difficult to characterise *alpestris* from *tricolor*, the characters named may be found in varieties of both species; the shape of the upper leaves may be the most unfailing one; in *alpestris* they are broadly ovate (fig. 7) or even with a cordate base, while *tricolor*'s are lanceolate (fig. 6). The two types used for the crossings were both extracted from a collection of seeds received from the Botanical Gardens in Brno, Czechoslovakia, viz. the blotched variety named above and a yellow, non-blotched one. Both of them had dark spot on the style.

Figs. 6—8. Flowers and leaves of 6: *tricolor typica* Line 504, 7: *alpestris velutina* (var. *polychroma*) and 8: *arvensis*.

V. arvensis MURR., n = 17.

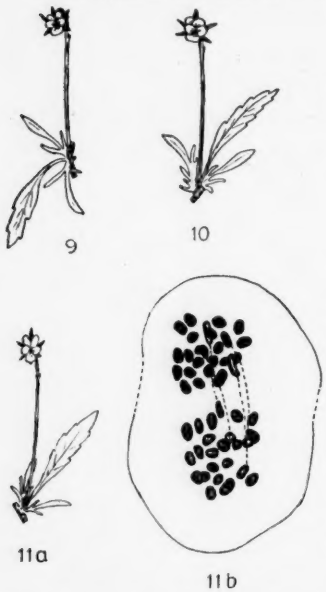
See description of types and variation in WITTROCK 1897 (many coloured plates) and in J. CLAUSEN 1922 and 1926. Characterised from *tricolor* and *alpestris* by its small flowers (petals smaller than sepals, see the two left flowers in the lower row of fig. 5 and the line drawing, fig. 8), its yellowish white flower colour (not *alba*) and the absence of labellum under the stigma; the stipules are lyrate or pinnatifid.

By the structure of the flower the species is autogamous and it is also strictly annual.

V. Kitaibeliana ROEM. et SCHULT., sens. lat.

Syn: *V. nemausensis* JORD. — *V. Kitaibeliana* resembles *arvensis*, but it is in all respects smaller, and especially its flowers are smaller and more closed. The petals of *arvensis* have spread-out and flattened limbs, while those of *Kitaibeliana* form a small cup-shaped corolla (see the four last flowers in the lower row of fig. 5). Just as in *arvensis* the flowers are yellowish white without labellum.

The species, although as to its content of types morphologically much more uniform than *tricolor*, comprises a number of chromosomal different types, namely with n = 7, 8, 12, 18 and 24 and possibly still more numbers. The following were used for crossings:



Figs. 9—11. Flowers and leaves of *9*: *Kitaibeliana* n = 7, *10*: *Kitaibeliana* n = 18, *11 a*: *nana* and *11 b*: heterotypic anaphase of *nana*, 2n = 24 + 24; for clearness sake are the two nuclear plates removed a little from each others (× ca. 1800).

n = 7, (fig. 9) a type from Caucasia sent from the Tiflis Botanic Gardens. A tiny type with rather long internodes; no spot on style.

n = 18, (fig. 10) also from Caucasia, courteously sent by Dr. G. WORONOFF. A stout and erect type, in its vegetative parts distinct from the preceding one but the flowers very similar; no spot.

V. nana DC., n = 24 (figs. 11 *a* and 11 *b*); the smallest of all the species and more tiny than *Kitaibeliana* n = 7. Its morphological characters are covered by the description of *Kitaibeliana*, except that the corolla is a little more open flowered. The seeds of this interesting type were collected on the island Jersey in the English Channel and kindly sent me by Dr. E. DRABBLE. Slight irregularities may take place during the progress of the meiosis, although it gives the impression of having a fairly constant chromosome number.

The group formed by the last mentioned species (*tricolor*, *alpestris*, *arvensis* and *Kitaibeliana* sens. lat.) contains the only annual and lowland species in the entire section; *alpestris*, indeed, forms a transition, being subperennial and subalpine. They have the widest distribution of all the pansies; *tricolor* and *arvensis* occupy Northern and Middle Europe and push forward into Western Asia; *arvensis* goes more southern than *tricolor*, which in the subalpine areas is supplanted by *alpestris*. *V. Kitaibeliana* in its distribution is more southern than the other ones, mainly Mediterranean to West-Asiatic; the most western type of it is *V. nana*, whose area is the surroundings of the Channel. The small-flowered species of this group, as *arvensis* and *Kitaibeliana*, are the only *Melanium* species without labellum under the stigma or at least with a very small one and are there-

fore also the only autogamous ones. All large-flowered species have this labellum, which prevents spontaneous selfpollination.

V. rothomagensis DESF., n = 17.

Syn: *V. hispida* LAM. Illustrations: WITTROCK 1897, plate XI, figs. 178—181. *V. rothomagensis* is a strictly local species from the calcareous tracts in northwestern France. The flowers (fig. 5, the middle flower in the upper row) are violet and remind about those of a large flowered *tricolor*, but *rothomagensis* is perennial, and the stems are more weak than *tricolor*'s. The upper leaves of *rothomagensis* are ovate with a cordate base (fig. 12), while the corresponding leaves of *tricolor* are lanceolate to linear-lanceolate. The most characteristic trait of *rothomagensis* is the straight, stiff and spreading hairs, with which the stems and the leaves and especially the margins of these are beset. They give the leaves and the stipules a ciliate appearance. The hairs of *V. Orphanidis* are more weak and also more dense than those of *rothomagensis*. With spot on style. The phylogenetic relationship of *rothomagensis* is not clear. Its position seems to be a little isolated, although it shows some relationship with *tricolor*, *arvensis* and *Battandierii*. The type used came from the Botanic Gardens of Rouen.

Figs. 12—14. Flowers and leaves of *12*: *rothomagensis*, *13*: *calcarata* and *14*: *lutea*.

V. calcarata L., n = 20

Illustration: WITTROCK 1897, plate VII, figs. 97—98. *V. calcarata* is a decidedly alpine and perennial species. *Calcarata* itself in strict sense is not much variable, but taken together with its near relatives *V. Zoysii* WULF., *Bertoloni* SALIS., *nebrodensis* PRESL. and the widespread *heterophylla* BERTOL. it forms a very variable group of near related and not easily defined species. Characteristic for *calcarata typica* are the very short internodes, the broad upper petals and the long, straight spur; the colour of the flower is violet in the type, but yellow in the var. *flava* and in *V. Zoysii*. The type used for the crossings was received from the Botanic Gardens of Gothenburg as *V. Bertolonii* DE SALIS. It is a rather typical *calcarata* (fig. 13), except that the spur is a little shorter and the petals not just as broad as in the *calcarata* proper, which is very difficult to cultivate.

V. lutea HUDS., n = 24.

This species may be related to the *tricolor-arvensis* group, but is perennial. It is subalpine and has a distribution from Scotland and Ireland through the Middle-European mountaineous districts to Austria and Hungary. The upper leaves are short ovate, and it has rather large yellow flowers, in some of the types saturated yellow flowers. The type used in the experiments belongs to the western subspecies, *elegans*

KIRSCHL.; it has long and thin, underground stolons but was received from Glasgow Botanic Gardens under the name of *V. tricolor.* The flower colour of it is bleached yellow, and it segregated types with violet petals (var. *amoena*) and some with the two upper petals velvety (*velutina* 1). The flower of this type (fig. 14) is not larger than that of *tricolor.* Characteristic are the dark, very furcate striæ on the three lower petals. It has a dark spot in front of style. It suffers from inbreeding, and was therefore difficult to maintain in cultivation, except by spontaneous pollination between different individuals in the Copenhagen Botanic Gardens. Fig. 5 (upper row, second from left) shows a flower of the var. *calaminaria* (LEJ.) also belonging to subsp. *elegans.*

The cultivated *V. lutea* (see figure in WITTROCK 1897, plate VI, figs. 80—81) probably originated from the eastern type, subspec. *sudetica* WILLD., which is more erect, more deep yellow and more large flowered. From crosses between the cultivated *V. lutea* and old cultivated types of *V. tricolor* took our garden pansies *V. Wittrockiana* GAMS (= *V. tricolor maxima* hort.) their origin.

V. Battandierii W. BECKR., the garden type with oscillating chromosome number, n = ca. 26—30.

Syn: *V. Pseudo-Munbyana* W. BECKR. The type used of this species is completely identical with the *V. Munbyana* described and shown in WITTROCK 1897, plate XI, figs. 173—177, and it was received from Muséum d'Histoire Naturelle in Paris under the name of *V. gracilis* SIBTH. et SM. This type is commonly grown in Botanical Gardens under different names, and it was apparently also used by KRISTOFFERSON (1923) for his crossings with *V. arvensis* and *tricolor.*

There has been some confusion as to the identity of this type. It is not identical with the North African (Algerian) species *V. Munbyana* BOISS. et REUT., which has much smaller stipules and is considerably smaller in stature than the Botanic Gardens type, being one of the largest pansies at all. The true *Munbyana* is by transitions connected with *V. heterophylla* BERTOL. (subsp. *ovatifolia* W. BECKR.), *V. gracilis* SIBTH. et SM. and *V. nebrodensis* PRESL. [var. *grandiflora* (GUSS.) CARUEL], all belonging in the group of *V. calcarata.*

The question now remains, if the type from the Botanical Gardens is the replica of any other wild growing type. BECKER first (1905) supposed this and identified it with a type collected in Algeria by REVERCHON 1896 (No. 192) under the name of *V. Munbyana*, by BECKER named *Battandierii.* Later (1925 b) BECKER thought the Botanic Gardens type to have no known wild growing representatives, and in order to distinguish it from the North African one he named the garden type *V. Pseudo-Munbyana.* The present author thinks this is to overemphasize the differences and has already (1927 b) drawn attention to the fact that types collected in cedar forest at Teniàt-el-Had in Algeria by C. M. POULSEN (1870) and by A. LETORNEUX (1888) very much resemble the types from the Botanic Gardens, also as to size and the characteristic shape of the stipules. BECKER himself drew this type to *Battandierii.* The writer, therefore, identifies the Botanic Gardens type as a varietas *hortensis* of the North African *Viola Battandierii* W. BECKR.

The type in question is strictly perennial, very vigorous and rich flowering. The leaves are large, ovate, and the stipules palmately lobed with a large foliaceous endlobe and a number of lanceolate side lobes (fig. 15 *a*). The peduncles are very long and very easily detached from the stem by a kind of joint. The flowers are large,

violet and with a short spur; the flower in the upper left corner of fig. 5 is of *Battandierii* and shows the characteristic triangular shape of the lower petal.

In the Copenhagen Botanic Gardens it constantly crosses with *rothomagensis*. It suffers from inbreeding and has to be maintained by open pollination. During the winter 1928—29 the original culture at the experimental field at Lyngby completely disappeared. This was so more to regret, because the old chromosomal count meantime had proved unsatisfactory, due to primitive fixation with CARNOY's fixative and staining with DELAFIELD's hæmatoxylin. Two or three homotypic metaphases

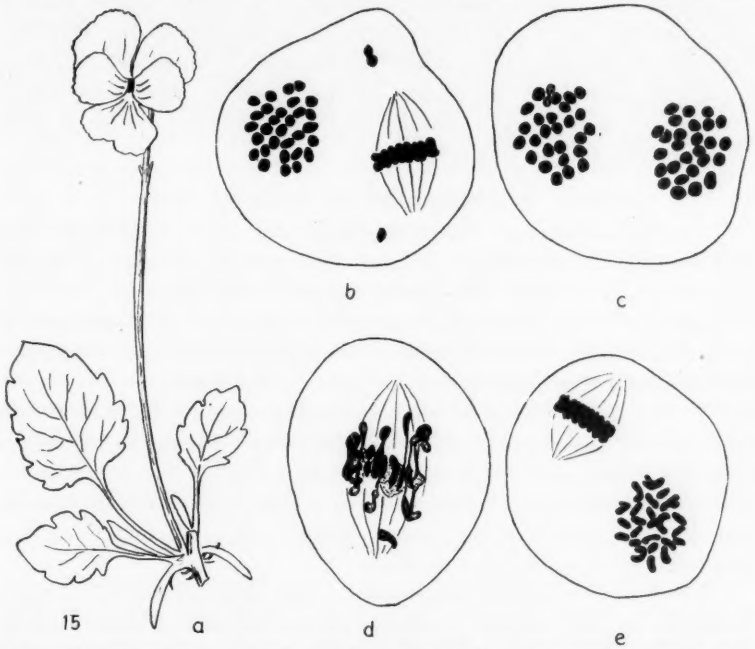


Fig. 15. *V. Battandierii*. *a*: flower, leaf and stipules; *b*—*c*: homotypic metaphases of pollen mother cells, n = 30, abt. 26 and abt. 27, in *b* two and in *c* one chromosome (divided, left) outside the nuclear plates; *d*—*e*: selfed individual (see text), *d*: heterotypic metaphase and *e*: homotypic metaphase, n = ca. 24—26 chromosomes of a long shape. (*b*—*e* × 1800.)

showed 30 chromosomes (fig. 15 *b*), but others not more than 26, 27 or 28, (fig. 15 *c*), and these last ones were thought to represent cut pollen mother cells with incomplete nuclei. In fact they were complete. In heterotypic metaphases of these old fixations the chromosomes appear conglomerated as if polysomes occur, but they are impossible to follow. From a selfpollination only one plant was obtained; it was very weak. Fig. 15 *d* shows the heterotypic metaphase from this plant with very long and irregularly disposited chromosomes, fig. 15 *e* a homotypic metaphase with 24—26 rather long, bent chromosomes. — The hybrids suggest that the gametes of the original *Battandierii* type generally carried not far from 30 chromosomes. It is thus the

Melanium species with the largest chromosome number known, but represents a cytologically irregular type similar to *Viola canina* (J. CLAUSEN 1931 b) although the type as judged from illustrations and herbarium specimens maintains itself morphologically.

III. MORPHOLOGICAL NOTES ON F_1. DESCRIPTION OF GENIC COOPERATION.

Instead of giving a detailed description on each F_1 it is thought better here to treat the individual characters collectively for a number of hybrids. This may prove useful for botanists, who observe supposed hybrids in nature. A description on the action of genes will also be given, which is necessary primarily for the complicated system of genes effecting colouring of flowers.

DURATION OF LIFE.

The *perennial* type acts as dominant over the *annual* one. This character was involved in the Crosses 1, 13, 14, 15 and 16 (see the diagram, fig. 1 or table 1). The perennial character apparently is a very complex one, and several genes may be responsible for it, because the species used in the experiments are perennial to a very different degree, representing a true gradation regarding this character.

V. cornuta and *Battandierii* are strictly perennial, the same is *lutea* on its natural habitats, but under the conditions, which the experiment field offers and probably also due to inbreeding, it is weak. On the experiment field *rothomagensis* lives 2—3 years, *elegantula, Orphanidis* and *alpestris* behave as biennials, although they are told to be true perennials on their natural habitats.

The F_1 hybrids here mentioned are not more perennial than their perennial parent: *tricolor* × *rothomagensis*, *arvensis* × *rothomagensis* and *tricolor* × *lutea* live two years at least. *Cornuta* × *elegantula* is still very vigorous after six years and the same is *tricolor* × *Battandierii*, although the *tricolor* type applied is strictly annual.

Hybrids between two perennials are also perennial: Cross 22, *lutea* × *Battandierii*, is still very strong and rich flowering after eight years lifetime, although its parents died (*Battandierii* probably due to winter conditions); Cross 4, *cornuta* × *orthoceras*, is also vigorous after four years, although it was impossible to keep *orthoceras* in culture.

F_1 of Crosses 6, *tricolor* × *elegantula*, and 7, *tricolor* × *Orphanidis*, were partly biennial just as their male parents.

In F_2 of the crosses named several types were segregated, of which a number had the general appearance of perennials and others the

character of annuals, but the plan of the experiments has not hitherto allowed a maintaining of the F_2 populations through a number of years in order to test the length of life of the segregated types. Annuals crossed with annuals invariably have given plants of annual type, in F_1 as well as in F_2.

Considering the evolutionary points of view, it should then seem more probable that the annual types originated from the perennial ones than *vice versa*, if they are of monophyletic origin at all. Annual violets are indeed the exceptions. Among the largely 500 *Viola* species recognised are no more than about five annuals, of which the four belong in the *Melanium* section in close relationship with *Viola tricolor*. They are: *V. tricolor*, *arvensis*, the *Kitaibeliana* group including *V. occulta* LEHM., *parvula* TIN. and related types, and finally *V. Rafinesquii* MUHL. from the Eastern United States of America. The fifth annual *Viola* species, viz. the East Asiatic *V. diffusa* GING., has the same number of chromosomes, n = 13, as *V. tricolor* (MIYAJI 1929), a number not found elsewhere among the violets. BECKER (1925) places *diffusa* in the *Nomimium* section, but morphologically it is a *Melanium* violet. MIYAJI (1929) makes suggestion as to the character of its leaves and stipules being similar to those of the *Melanium* section, and its structure of style (J. CLAUSEN 1929, fig. 44) sooner places it in the *Melanium* than in the *Nomimium* section. This brings all the annual species into one section. It would be of interest to try a crossing between *diffusa* and *tricolor*.

SIZE OF FLOWERS.

The character *small flower* (i. e. petals shorter than the sepals) is prevalent over the *large flowering* type (petals larger than sepals). The Crosses 9, 10, 11, 12, 16 and 21 all have a large and a small flowering parent. All F_1's were small intermediates as to size (see Table 3).

F_1 of Cross 16 showed some variation in flower size with a mean of abt. 7,5 mm. for the two upper petals; the other crosses had fairly constant flower size. For the three first named hybrids the pure white *alba* type of *tricolor* was applied. The two upper petals of its flowers are not 13, but only 10 mm., indeed, but this is due to the fact only that it contains none of the basal genes *A* for anthocyanin flower colours; as soon as one gene *A* is added, the flowers attain full size. As all F_1's carry *A* (from their small flowered parent) the size value attributed to the *tricolor* parent must be that of the anthocyanous coloured type.

TABLE 3. *Length in millimeters of upper petals in F_1 hybrids and in their parents.*

small flowered parent			F_1			large flowered parent		
	n=	mm.	Cross no.	actual length in mm.	calculated mean length in mm.	mm.	n=	
Kitaibeliana...	7 18	3,0 3,5	10 11	6 5	8,0 8,25	13	13	*tricolor*
nana	24	2,5	12	6,5	7,75			
			21	6,5	7,75	13	24	*lutea*
arvensis	17	5,0	9	7	9,0	13	13	*tricolor*
			16	7,5	9,0	13	17	*rothomagensis*

There may be several genes affecting the flower size, some increasing it, others decreasing it, but the most superior one in its effect seems to be an inhibiting gene, *F*, present in the small flowered species (J. CLAUSEN 1926). Large flowering plants are then *ff* and may in addition have some minor genes, positively increasing the flower size, while real small flowered species have no such ones. Due to the interaction of these genes, F_1 is not real small flowered but small intermediate. The hexaploid *V. Kitaibeliana* n = 18 may have at least two inhibiting F_1-genes as suggested from the very small flowers of F_1 and from the segregation in F_2. Diploid *Kitaibeliana* n = 7 seems to have a less inhibiting effect than hexaploid *Kitaibeliana* upon the flower size of F_1, probably because it has no more than one principal inhibitor.

Otherwise the chromosome number itself seems to be of no effect in stamping the type; which species shall be the predominant one seems to be due to the effect of their genes only, not to their amount of chromosomes. Small size of flowers is prevalent as well in Cross 10, where it was introduced by the parent with the smallest number of chromosomes as in Crosses 9, 11 and 12, where the parents with the largest chromosome number introduced small flower size. KRISTOFFERSON (1923) measured length of petals in F_1 and F_2 of the Cross *tricolor* × *arvensis* and found similar relations.

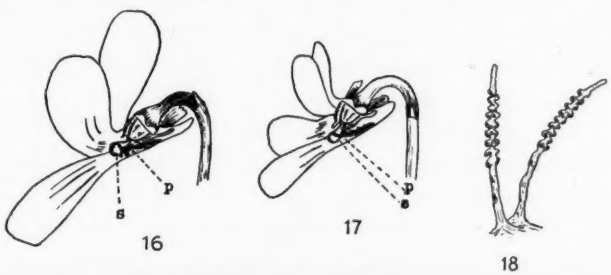
SIZE OF LABELLUM.

All large flowered species of the *Melanium* section have a minute *labellum* on the front side of the style under the stigma, while

the few species with small flowers, previously mentioned, have no such one.

This easily overlooked character is a significant one in the biology of the flower, as it practically determines if the species is capable of spontaneous selffertilisation or not. When present, it prevents the pollen from falling into the stigma.

Although presence or absence of labellum seems to be the principal factor in determining if the species shall be a hercogamous or an autogamous one, this outcome is influenced also by other mechanisms, which likewise seem to be correlated to labellum and flower size. The pollen, which fall from the anthers, are retained by the *pollen magazine* (figs. 16—17) a variably shaped groove on the proximal part of the spur



Figs. 16—18. Flowers cut through in order to show the interrelation between stigma (*s*) and pollen magazine (*p*); *16* is from *tricolor* (with labellum under the stigma and closed pollen magazine); *17* is from *arvensis* (no labellum, open magazine); *18*: hairs from the pollen magazine (enlarged).

bearing petal. The walls of this groove are formed by peculiar hairs (fig. 18) irregularly fitted with wart-like processes on their surface, serving to retain the pollen — either for later removal by insects hunting the nectar secrated by the glandular projections from two of the anthers — or for a gradual dropping them into the unsheltered and somewhat backwards turned stigma of the small flowered species. Presence or absence of labellum, shape and relative length of the pollen magazine and direction of stigma seem to be the principal agencies determining the mode of pollination.

There seems to exist a mechanism keeping together all these characters in two blocs, namely (1) large flowers, stigma directed somewhat forwards sheltered by a labellum and placed outside a pollen magazine, which is closed in front (fig. 16) and (2) small flowers, with stigma directed downwards and somewhat backwards, its opening

pressed down in the pollen magazine, which forms a funnel-shaped channel open in front (fig. 17). It is for these species a lucky case that small, inconspicuous flowers coincide with a structure, which facilitates selfpollination. By the cross *arvensis* × *tricolor* (J. CLAUSEN 1926, pp. 82—85) it was shown that the mechanism, which keeps »small flowers» and »no labellum» together is a genetical linkage, apparently because the inhibitors for flower size and for labellum are located in the same chromosome at some distance from each others.

In crosses between species without labellum or with an extremely minute one and species with distinct labellum F_1 has almost no labellum (compare also KRISTOFFERSON 1923, CLAUSEN 1926), and the principal inhibitor, *B*, for labellum is thus prevalent in its effect, just like the inhibitor for flower size. In some cases F_1 is more or less hercogamous, in other cases autogamous (compare KRISTOFFERSON 1916) according to the factors, which in each special case influence the biology of the flower.

DIRECTION OF LATERAL PETALS.

The direction of the lateral petals is a valuable character in the classification of larger taxonomic units. All the violets of the *Nomimium* section have downwards turned lateral petals and all *Melanium*

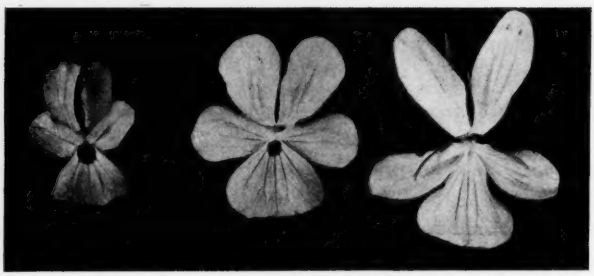


Fig. 19. From left: flowers of *elegantula*, *cornuta* × *elegantula* F_1 and *cornuta*. Portrait film without filter in order to show extension of bright yellow eye.

violets save the two exceptional species *V. cornuta* and *orthoceras* have upwards turned side petals. This character is involved in the Crosses 1 and 5. F_1 is intermediate with »horisontal» lateral petals as fig. 19 (*cornuta* × *elegantula*) shows. This behaviour in the cross was noted already by WITTROCK (1897, plate 7, figs. 89—90 and plate 9, figs. 125—126).

INTENSE YELLOW EYE.

V. cornuta and *orthoceras* deviate also from all other *Melanium* violets by not having the intense yellow eye on the proximal part of the lower petal. F_1 of the Cross 1 above had a yellow eye just as intense as in the *elegantula* parent, although its extent was smaller than in *elegantula*. Thus this character is dominant as to intensity but intermediate regarding extension. Fig. 19 is from a photograph on portrait film and shows very distinctly the difference of the yellow between the two parents and the F_1.

HAIRINESS.

Two hairy species are included in the crossings, viz. *V. rothomagensis*, hispid by rather dense, straight and stiff, spreading hairs and *Orphanidis*, very densely hirsute by more soft hairs.

The *hispid rothomagensis* was used in the Crosses 13 and 16 with *tricolor* and *arvensis* (almost glabrous with few appressed hairs) as the other parent. F_1 was somewhat intermediate but conspicuously hispid, although the cilias were shorter and more remote than in *rothomagensis*.

The *hirsute Orphanidis* was used in the Crosses 2, 5 and 7, with *elegantula*, *cornuta alba* and *tricolor* respectively. *V. elegantula* and *cornuta alba* are almost completely glabrous and F_1 of Crosses 2 and 5 were minutely puberulent on young stems and had remote, short, appressed hairs on the leaves. F_1 of Cross 7 with *tricolor* had rather few and remote hairs being not much more hairy than *tricolor* itself. F_2 and later generations contained never a plant nearly as hirsute as *Orphanidis*; some of them were more or less puberulent, but never hirsute. This may be due to elimination of certain types, as F_1 was very sterile.

Thus the *rothomagensis* hispid type of hairiness gives the impression of being dominant or at least prevalent, while the *Orphanidis* hirsute type seems to be recessive.

FLOWER COLOUR.

Anthocyanin. The violet, red and more or less dark velvety flower colours depend upon the presence of at least one gene of the polymeric series (series of multiple genes) A_1, A_2 and A_3 for anthocyanous colour in stems, leaves and flowers. They seem to be basal genes for flower colour also; they are dominant and give full effect in a single dose but may be present up to six times; the species with the largest chromosome number have also more *A*-genes.

If all *A*-genes are absent, the stem is pure green without anthocyanin and ordinarily the flower then is also pure white (*alba*). It may, nevertheless, be converted into *dilute mauve* by the action of a gene, *D*, which is hypostatic to all *A*-genes but dominant, when its action is not covered by *A*. This gene is present in *V. arvensis* (J. CLAUSEN 1926, p. 107) and in *alpestris*.

All plants recessive for *A* are smaller and have also smaller flowers than those with anthocyanin. It does not make any difference in this respect, whether they have *D* or not. *aaD*-plants may have a dilute mauve tinge on the stems, but this colour is different from the dark anthocyanous *A*-colour.

Genes of the *A*-series were analysed in the Crosses 1, 5, 6, 7, 8, 9, 11 and 13; 10 and 12 are not yet carried further than to F_1.

Velvety colours (*velutina*). The flower colour produced by the action of *A* alone without any other genes is really black velvety and extremely dark (fig. 5, middle row), but all wild species have a series of dominant and partial epistatic genes (M_1 to M_5) which gradually modify this dark colour to violet; M_1 is the most superior and epistatic of these, able to change black to violet by almost one step (see J. CLAUSEN 1930 a, pp. 349—355). The flower colour contingent upon A is thus intensified by the absence of genes of the *M*-series. Dilute mauve *aaD* cannot become *velutina* or dark by absence of the *M*-genes.

Reddish (*rose*) *flower colour*, *rr*, is recessive. It demands the presence of one of the genes *A* for anthocyanous cell sap and it is changed to colours of the violet series by its dominant alternative, *R*, probably converging the reaction of the cell sap. The dilute colour contingent upon the presence of *D* exists also in both a dilute mauve (*aaDR*) and a dilute rose (*aaDrr*) edition. Reddish can be intensified to reddish *velutina* (velvet) by the absence of genes of the series M_1 to M_5. Reddish or rose is often present in *V. elegantula* and was found in wild growing populations of *tricolor maritima*. It was analysed in Cross 6 and previously (J. CLAUSEN 1926) in Cross 9 (*tricolor maritima rosea* × *arvensis*) and three varietal crosses of *tricolor*. In all cases it was found to be recessive to violet (*AR*); *V. tricolor hortensis* and probably also *V. arvensis* were found to possess two polymeric *R*-genes.

Intense yellow flower colour, *LL*, is most intense on the spur-bearing petal (the lower one); it is namely epistatic to the violet colour contingent upon the action of *AMR*, and as this is most strong in the upper petals, *LL* cannot here suppress it to more than yellowish white; in spring time, when the plants are more anthocyanous, the upper petals

of *AMRLL*-flowers are often brownish purple. The *L*-gene occurs in some types of *V. tricolor* (J. CLAUSEN 1926), it is characteristic of most types of *alpestris*, it is present in *arvensis* but its effect is here covered by a bleaching gene (*W*), and this seems to be the case also with all *Kitaibeliana* types; the 18-chromosome type, at least, contains it. Finally, it is present in *Viola lutea*, but the type used in the crossing apparently also has a bleaching gene (called *Pal*) of a less bleaching effect than the *W*-gene.

It is obvious that a number of other genes govern the extension of yellow, for in some cases only the lower petal is intense yellow, the upper ones being more or less violet, while in other cases the *LL*-genes bleach the upper petals to yellowish white, and in still other types also they are intense yellow. A similar difference can be seen in the reaction of the heterozygotic type, *Ll*, which, if it is a pure *tricolor* type, begins as yellowish white, when the flower is just opened, but by and by the upper petals darken to pale violet, so that whitish and violet flowers may be present on the same plant, what is often noted on specimens occurring in nature; in this species the heterozygote can safely be distinguished from the violet and the yellow homozygotes. In Cross 8, *alpestris* × *tricolor*, on the other hand, the *L*-gene from *alpestris* is not able to bleach the violet colour of *tricolor*, except when twice present; the heterozygote, *Ll*, must therefore be classified together with the homozygotic violet coloured *ll*-individuals. When violet colour is intensified by absence of the *M*-genes, *LL* has no visible effect on the upper petals. The gene *L* enters in the Crosses 7, 8, 9, 11, 14 and 22.

Yellowish white flower colour is due to the dominant or at least prevalent bleaching gene, *W*. Its bleaching of violet or rose of the *A*-series is not complete, except when together with *LL*. The gene *W* is present in the small flowered species *V. arvensis* and *Kitaibeliana* sens. lat. It may be present also in the large flowered *V. rothomagensis*, although the genetical basis of colouring of flowers in this species has not been fully cleared up yet.

F_1 of all crossings, of which one parent is yellowish white, are rather whitish flowered, although the older flower may have a tinge of pale violet. This has been found for the Crosses 9, 11, 12 and 16. F_1 of Cross 10 (*Kitaibeliana* n = 7 as one parent) behaves in a different way, as it is rather violet of flower colour, although *Kitaibeliana* itself is whitish, at least during mid-summer. Probably this *Kitaibeliana* type has no *L*-gene.

A superior gene for violet flower colour. Apart from the two types

of genes giving more or less violet flowers, namely those of the *A*-series and the hypostatic dilute colour *D*, still a third type of violet flower colour may occur caused by a gene, *V*, which is epistatic over intense yellow, *LL*, (this again being epistatic over the basal violet flower colour, *AMR*, of the pansies). The play between these different genes for violet is very complicated. The gene *V* has been shown to exist in *V. Orphanidis* (Cross 7), and the violet flower colour of *V. rothomagensis* may depend upon this gene also (in cooperation with *W* and *L*), because the results of Cross 16 would otherwise be inexplicable. The *V*-gene is probably also present in an *alpestris* type received from Mount Bistria (Scardus) in Yugoslavia under the name of *V. elegantula*. It has 13 pairs of chromosomes and segregated an intense yellow type similar to the so-called *V. macedonica* BOISS. et HELDR. (= *V. alpestris*).

TABLE 4. *Some flower colours in Melanium violets and their gene symbols.*

$a_1a_2a_3$	*dd*	*alba*
	D. . . . . *R*.	dilute mauve
	D. . . . . *rr*	dilute rose
	dd . . . . . *LLww*. .	*alba*-yellow (*aaLl* = *alba*)
	D. . . . . *LLww*. .	*alba*-yellow with dilute mauve upper petals
A. . . .	. . $m_1m_2m_3m_4m_5R.llww$. .	black velvet (*velutina* 5)
	. . $m_1m_2m_3M_4$. . *R*. *llww*. .	violet *velutina* 3 (= *tricolor hortensis*)
	. . $m_1m_2m_3M_4$. . . . *LLwwvv*	yellow *velutina* 3
	. . m_1M_2 . . . . . *R*. *llww*. .	violet *velutina* 1
	R. *llww*. .	violet (= *tricolor typica*)
	rr llww. .	rose
	. . *Llwwvv*	whitish to pale violet (sometimes violet)
	. . $M_1(M_2M_3M_4M_5)$ . . *LLwwvv*	intense yellow (= *V. lutea* and *alpestris*)
	. . *LlWwvv*	whitish pale violet
	. . *LLWWvv*	yellowish white (= *arvensis*)
	. . . . . *V*.	violet (= *Orphanidis*, possibly also *rothomagensis*)

Table 4 gives a survey on the most significant types of flower colour and their formulæ.

DARK SPOT ON STYLE.

In Cross 9, *arvensis* × *tricolor*, and in varietal crossings of *arvensis* and of *tricolor* (J. CLAUSEN 1926) it was found that the inheritance of the trivial character of a small triangular, dark spot in front of the style under the stigma was governed by an intricate cooperation of a number of genes, of which some were positive genes for spot and some

of them were inhibiting genes for certain of these, not for all of them. One gene for spot, at least, needed a complementary gene for its realisation, and the inhibiting genes were also complementary as to their effect. There was some evidence suggesting that the different genes for spot, reacting in different ways with the inhibiting and complementary genes, belonged to a multiple series of allels. The interaction of inhibiting genes with positive genes for spot caused the character of spot sometimes to behave as a dominant, sometimes as a recessive. When the inhibitors are weak in their effect a safe classification of the types is impossible. *V. Orphanidis* has such weak inhibitors together with positive genes for spot. In all other specific hybrids the spot character behaved as contingent upon a single dominant gene; namely in the Crosses 8, 10 and 11 (spot gene from *tricolor*) and Crosses 13 and 16 (spot gene from *rothomagensis*).

IV. NOTES ON CYTOLOGY AND FERTILITY OF F_1.

It is a matter of course that it will be impossible to enter into any exhaustive description of the cytological investigation on each of the many hybrids, how tempting it might be, because almost each one shows some interesting detail. But so much barren philosophy concerning the meiotic behaviour of assumed hybrids and species of assumed hybrid origin has been published during the later years that it might be worth while to have a short description on the behaviour of a number of hybrids, whose parents are known.

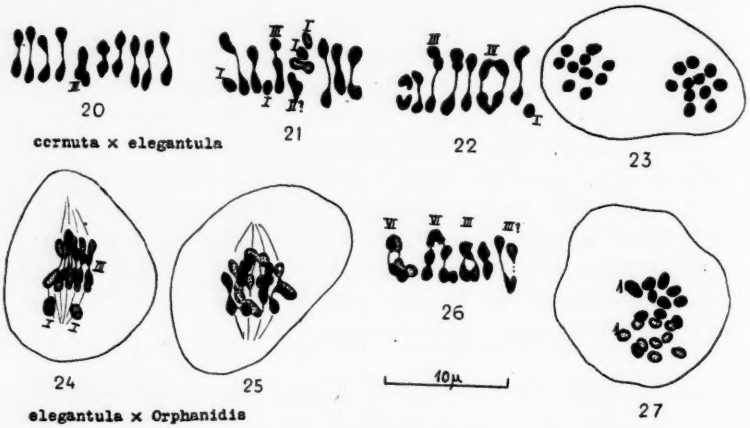
Cross 1: *V. cornuta*, $n = 11 \times$ *elegantula*, $n = 10$.

Heterotypic metaphases (figs. 20—22) show only few univalents (from one to six), but trisomes and tetrasomes are often present. One bivalent is often found lying »horisontal» (fig. 21). The parents show normal conjugation of 11 and 10 pairs without polysomes. Irrespective of multivalents and univalents the distribution of the chromosomes seems to proceed not too irregular, as homotypic metaphases (fig. 23) look rather normal apart from the fact that the number of chromosomes cannot be the same in both daughter nuclei. Of 16 homotypic metaphase plates there were 11 chromosomes in nine and 10 in the seven, and no elimination was observed.

Cross 2: *V. elegantula*, $n = 10 \times$ *Orphanidis*, $n = 11, 10$.

Some *Orphanidis* types throw gametes with only 10 chromosomes as previously demonstrated (J. CLAUSEN 1930 b). The plant investigated

was a spontaneous hybrid arisen after free (uncontrolled) pollination of *elegantula* in the field. Fig. 28 shows the flower of the hybrid



Figs. 20—27. Meiosis of Cross 1 and 2. When, in the following, nothing otherwise is stated, the figures show pollen mother cells (pmc.). Roman numerals in the figures indicate the number of chromosomes in the conjugated or single units. Abbreviations: het. = heterotypic, hom. = homotypic, met. = metaphase, ana. = anaphase. — The magnifications are all × abt. 1800 (see scale referring to all chromosomal figures). — Figs. *20—23*: Cross 1; *20—22*: het. met., *22* not complete; *23*: hom. met., n = 10, 11. — Figs. *24—27*: Cross 2; *24—26* show het. met. with mono- and polysomes and irregular chains of chromosomes (fig. 25), *24* is probably not complete; *27*: het. ana., n = 10, 10, an unequal pair (marked 1) present.

between *elegantula* (left) and *Orphanidis* (right). The parental *Orphanidis* pollen carried only ten chromosomes.

Fig. 28. From left: flowers of *elegantula*, *elegantula* × *Orphanidis* F_1 (Cross 2) and of *Orphanidis*.

Characteristic for the heterotypic metaphase in the hybrid (figs. 24—26) is a *multivalent association* of chromosomes into open chains, but not in regular zig-zag (fig. 25); monosomes are rare, but on the other hand are hexa-, penta-, tetra-, tri- and ordinary disomes common. Fig. 26 shows the chromosomes of a nucleus in which only one bivalent was present. Rings of chromosomes are rare, and trisomes

form either a straight chain or a V as in fig. 24. The distribution of the chromosomes during the anaphase is surprisingly regular as regards their number at least. In most cases the daughter nuclei receive 10 chromosomes each (fig. 27). Very often a considerable difference between the members of one pair can be noted (see the two marked 1 in fig. 27).

V. Orphanidis shows occasionally trisomes and tetrasomes, at least the cytologically aberrant types do so (J. CLAUSEN 1930 b). But the polysomic arrangement observed in this hybrid far exceeds the sporadic irregularities in *Orphanidis*. One should think that many errors in the distribution of the chromosomes would occur by such a primitive and irregular mechanism of distribution, resulting in duplication and omission of single chromosomes ordinarily belonging to one of the two sets of ten. F_2 has not been seen yet but the fertility of F_1 was not bad, indeed, as abt. 250—300 seeds were obtained from each plant.

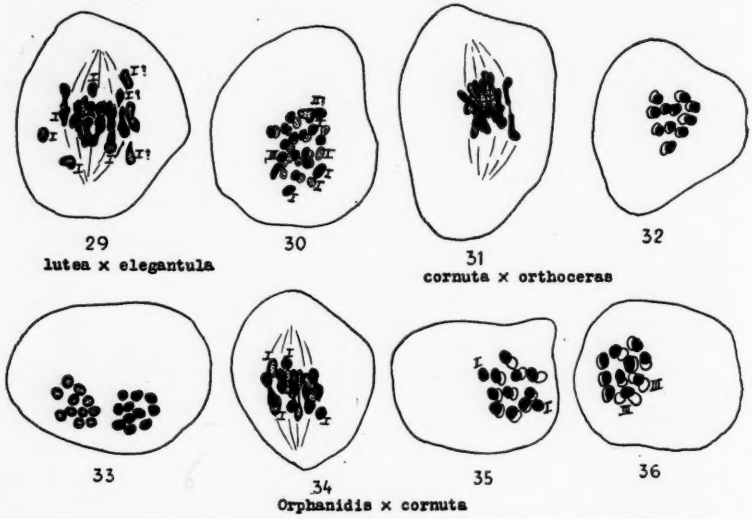
Cross 3: *V. lutea*, $n = 24 \times$ *elegantula*, $n = 10$.

The pollen mother cells of this hybrid do not stain so well as in the preceding one, the chromosomes are small and the counting difficult. Characteristic for this hybrid is a certain amount of *autosyndesis*, apparently of the *lutea*-chromosomes. If all *elegantula*-chromosomes paired with *lutea*-chromosomes, fourteen would be left unpaired. No more than from four to six univalents are observed in most pollen mother cells, and heterotypic metaphases seen in polar view show not more than abt. 19 units; 13—15 bivalents may be counted in addition to 4—8 univalents (figs. 29—30). In some cells chains of chromosomes appear. The fertility of this hybrid was poor (50—100 seeds per plant), only very few of the seeds germinated and the plants did not thrive well at all. Genetic analysis, therefore, was impossible.

Cross 4: *V. cornuta*, $n = 11 \times$ *orthoceras*, $n = 11$.

Characteristic for this hybrid is that the conjugation between the chromosomes seems just as complete as in the parent species. Eleven bivalents are observed during heterotypic metaphase and anaphase (fig. 31—33). Rarely an anaphase may show 12 chromosomes instead of 11; a single lagging chromosome may also be observed, but the main impression of the cytological behaviour is that of a pure species. The fertility is rather good, but the seeds germinate badly (12—20 %). F_2 segregated for minor characters only, and the parents are morphologically also very alike. Fig. 4 shows the type of F_1, which is rather

luxorious. It is evident that the two species are very near related, also as to their chromosomal and genetical constitution.



Figs. 29—36. Meiosis in Crosses 3, 4 and 5. *29—30*: Cross 3; het. met.; *29*: $13_{II} + 8_{I}$ (?); *30*: abt. $1_{III} + 13_{II} + 5_{I}$ (autosyndesis). — Figs. *31—33*: Cross 4, het. met. in side- and polar view and hom. met.; regular, 11_{II}. — Figs. *34—36*: Cross 5, het. met.; *34*: four univalents; *35*: $10_{II} + 2_{I}$; *36*: $8_{II} + 2_{III}$.

Cross 5: V. Orphanidis, n = 11, 10 × cornuta, n = 11.

The *Orphanidis* parent was V. 917—1, which had 2n = 21 chromosomes (J. CLAUSEN 1930 b). Consequently some of its gametes carried 11 and some 10 chromosomes. Of the five F_1-plants resulting from the crossing, two had 2n = 21 and three 2n = 22 chromosomes. There were no difference in morphological appearance or in the mode of meiosis of the two chromosomal types. The pollen mother cells gave the impression of being in good condition; the divisional pictures were clear, and the chromosomes stained well.

The conjugation in the plants with 22 chromosomes ranged from 8 to 11 bivalents with a corresponding number of univalents; some of the chromosomes were more or less clearly arranged into trisomes or tetrasomes (figs. 34—36). The plants with 21 chromosomes behaved in the same manner, but their largest number of bivalents was 10. The distribution of the chromosomes to the gametes was at random, as table 5 shows.

TABLE 5. *Distribution of chromosomes to the gametes during meiosis of Cross 5, n = 10 × n = 11 (2n = 21).*

Number of chromosomes	8	9	10	11	12	total
number of nuclei counted.....	1	3	18	17	1	40

Elimination or splitting of one or a few univalent chromosomes occurred in some cells. The fertility was rather good but only 10—20 per cent of seeds germinated. The F_2-plants as well as the F_1's were weak but a fairly good Mendelian segregation was obtained. No doubt *V. Orphanidis* must be considered one of the most near relatives of the taxonomical isolated *V. cornuta*, whether judged from the morphological appearance of the parents, or from the degree of chromosomal conjugation, the condition of the pollen mother cells and the fertility of F_1.

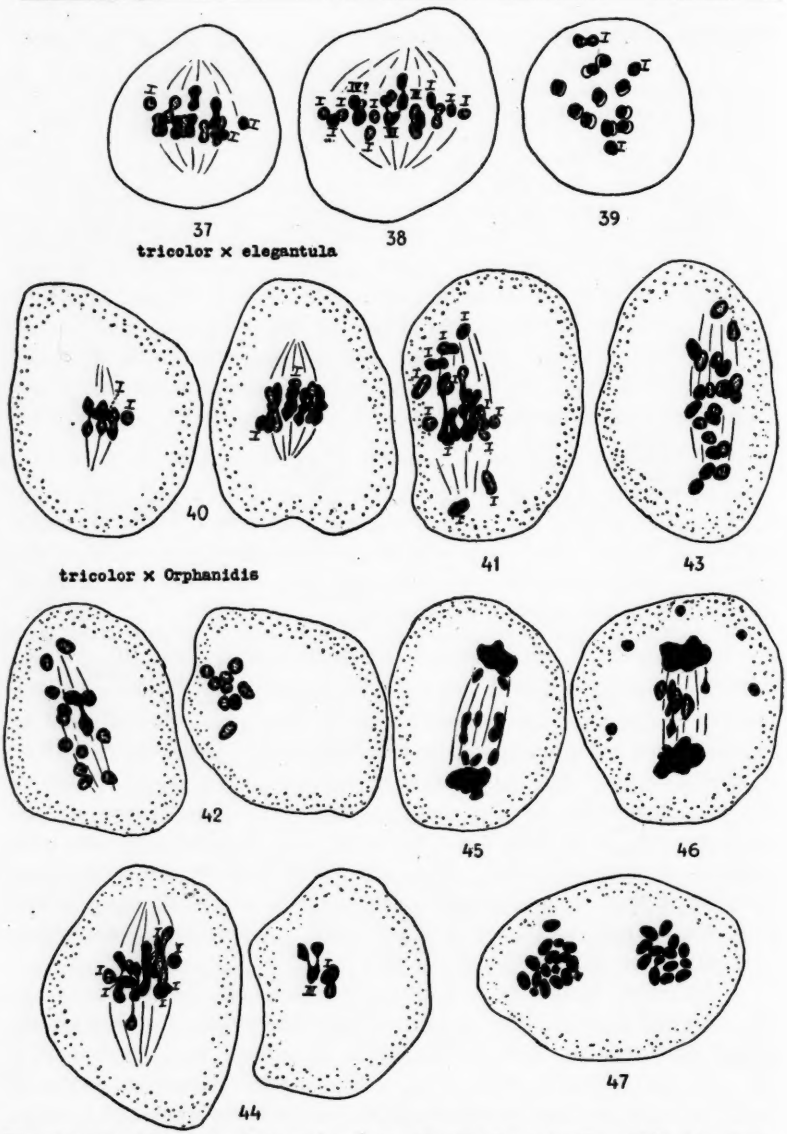
Cross 6: *V. tricolor, n = 13 × elegantula, n = 10.*

The meiotic divisions in the pollen mother cells are good looking, and the cells in a good condition. From 8 to 10 bivalents are formed, and polysomes (II, IV, VI) occur considerably often (figs. 37—39). The univalents range from three to seven. Fertility very poor, abt. 30 seeds per plant, and germination even poorer.

Cross 7: *V. tricolor, n = 13 × Orphanidis, n = 11, 10.*

The *Orphanidis* parent was 917—1 with 2n = 21 chromosomes, the same as in Cross 5. Consequently some F_1-plants had 23 and some 24 chromosomes according to whether the *Orphanidis* pollen carried 10 or 11 chromosomes. The conjugation of chromosomes was more *variable* in this cross than in the other ones, ranging in the same 24-chromosome plant from $10_{II} + 4_I$ (fig. 40) to $1_{II} + 22_I$ (fig. 42); probably also cells with 24 univalents occur. The cells with almost no conjugation were found together in one pollen sac, but even within the same pollen sac a great range of variation was observed. A great many observations were made on different plants of this cross.

The pollen mother cells appear well nourished and stain well, but the marginal zone of them contains numerous minute drops or grains, which stain with a colour similar to the nucleolus. It is a well known fact that the pollen mother cells of plants, which are in a less good condition, may contain nucleolar material outside the nucleus. In some cases a few larger nucleoli may be present in the plasm, in other cases more small nucleoli are scattered often most dense in the marginal zone (fig. 46); the many minute dots in the pollen mother cells of this



Figs. 37—47. Meiosis in Crosses 6 and 7. *37—39*: Cross 6; *37* and *39*: $10_{II} + 3_I$; *38:* abt. $1_{VI} + 1_{IV} + 1_{III} + 1_{II} + 8_I$. — *40—47*: Cross 7; *40—44*: het. met. with varying number of univalents, *42* and *43* (not complete) with almost no conjugation; *45—46*: het. ana. with splitting univalents; *47*: hom. met., n = abt. 15 (+ one outside the group) and abt. 14. This cross has many minute grains or drops in the plasm of its pmc. and also extranuclear nucleoli (fig. *46*).

hybrid, shown in the figures, may be of a similar nature. The writer observed dots just of a similar appearance in a non-hybrid *Phaseolus*. If stained with HEIDENHAIN's hæmatoxylin the larger extra-nuclear nucleoli are often mistaken for detached chromosomes, even by investigators, which should be regarded as trained cytologists. Although this condition may be most common in hybrids, it is also found scattered elsewhere in non-hybrid plants.

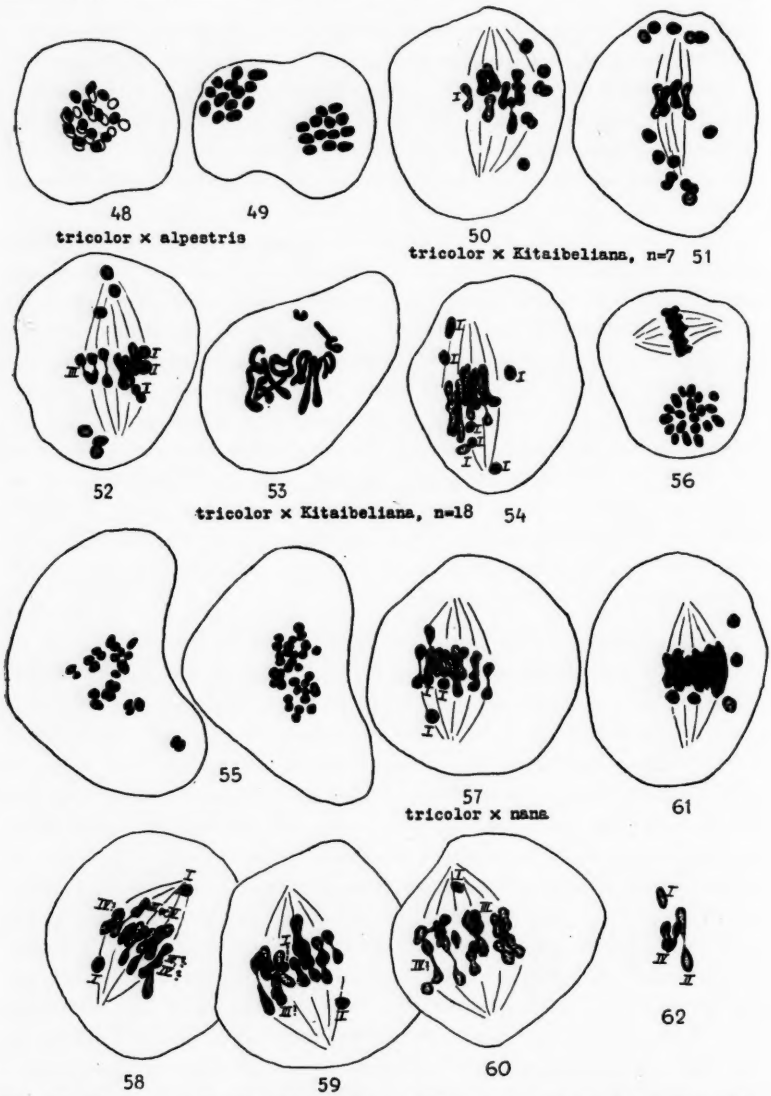
Some few trisomes and probably also some less typical tetrasomes (attached bivalents) are observed in some pollen mother cells of the hybrid *tricolor* × *Orphanidis*, but polysomes are much more rare in hybrids of *Orphanidis* than in hybrids of *elegantula*. The figures 40—44 show heterotypic metaphases; in heterotypic anaphases (figs. 45—46) some of the univalents may be seen dividing, giving rise to homotypic nuclear plates with an increased number of chromosomes; fig. 47 shows such a pollen mother cell containing 15 chromosomes in one and 15 to 16 in the other nuclear plate together with one detached; some of the chromosomes here are very small (univalents recently divided). Elimination of chromosomes is not uncommon, and the formation of dwarf nuclei gives rise to pentads, hexads etc. Fertility and the germination rate of the seeds was poor.

Cross 8: *V. tricolor*, $n = 13$ × *alpestris*, $n = 13$.

There is a slight tendency to univalency of some few chromosomes in this hybrid, and figures showing univalent chromosomes (of which a number splitting) were previously published (J. CLAUSEN 1927 b, figs. 65—66). Such irregularities are, nevertheless, exceptions and occur in certain plants and crossings only; most hybrid plants show regular meiosis (fig. 48), in which only a loose conjugation or a not quite parallel placement of the thirteen bivalents reveals the hybrid nature of the plant in question. Heterotypic metaphases in side view may show univalents and anaphases lagging chromosomes, just as also dwarf nuclei occur occasionally. Extranuclear nucleoli were observed in the plasm of almost invariably all pollen mother cells. Apparently nearly all gametes receive 13 chromosomes (fig. 49). The fertility was extremely good, the F_1-plants gave from 1500—2000 seeds each. The conditions in this cross remind about those in Cross 4, and the two species or subspecies must be regarded as very closely related.

Cross 9: *V. tricolor*, $n = 13$ × *arvensis*, $n = 17$.

In heterotypic metaphases from 11 to 13 bivalents and correspondingly from 8 to 4 univalents are formed. No clear instances of



Figs. 48—62. Crosses 8—12. *48* het. met. and *49*: hom. met. of Cross 8, regular, 13_{II}. — *50—52*: Cross 10, het. met. with 6_{II}, 4_{II} and $4_{II} + 1_{III}$, respectively, in addition to univalents. — *53—56*: Cross 11; *53*: long, semi-prophasic chromosomes, no nuclear membrane; *54*: het. met., at least 7_I; *55*: het. ana., no reduction; *56*: hom. met., $n = 20$. — *57—62*: Cross 12, het. met., autosyndesis; *57* with three, *58* and *59* with two, *60* with probably only one and *61* with five univalents in addition to some tri- and tetrasomes (*58—60*); *62*: tetra-, di- and monosome from the same cell.

trisomes or tetrasomes were found, but the arrangement of bivalent chromosomes in some heterotypic metaphases may indicate a slight secondary attraction between bivalents or between bivalents and univalents. The present writer is not in the possession of frankness enough to postulate that such slight and uncertain association of chromosomes be evidence enough of a true polysomic arrangement. DARLINGTON and MOFFETT (1930 b) appear to be inclined to see polysomic arrangement, not only in their own slides, but also in the figures of material, which they are not familiar with. But there is a great difference between the true polysomic arrangement, which is seen in some other *Viola* hybrids and also in cultivated races of *Pyrus* (DARLINGTON and MOFFETT 1930) and the slightly diverging arrangement of gemini in hybrids as *tricolor* × *arvensis*. Some allowance for occasional placement of two gemini very close to one another must also be admitted.

Elimination of one or two chromosomes is very common in *tricolor* × *arvensis* and the number of chromosomes has therefore a tendency to decrease during later generations. The fertility is very good and it gives from 500 to 2000 seeds per plant. This hybrid was previously dealt with in details (J. CLAUSEN 1926), but it is here mentioned for a comparison.

Cross 10: *V. tricolor*, $n = 13 \times$ *Kitaibeliana*, $n = 7$.

No more than six bivalent chromosomes were observed in heterotypic metaphase of this hybrid; in this case, which seems the most common, 8 univalents were present. But the combinations $5_{II} + 10_{I}$ and $4_{II} + 12_{I}$ were also often met with (figs. 50—52). A trivalent was observed in one cell (fig. 52). The pollen mother cells were not in a well nourished condition, but the chromosomes stained well and gave comparatively clear pictures. The hybrid was almost completely sterile, giving from none to 30 seeds per plant in spite of a good vegetative development and abundant flowering.

Cross 11: *V. tricolor*, $n = 13 \times$ *Kitaibeliana*, $n = 18$.

The pollen mother cells of this hybrid were poor-looking, and it was impossible to account for all chromosomes during the rather irregular meiosis. The nuclear membrane can dissolve before the nucleolus, and even before the chromosomes arrive to the typical diaphase with short chromosomes (see fig. 53). The number of bivalents in heterotypic metaphase could not be counted with certainty, but may be calculated from the number of univalents, which are much more easily counted. Seven univalents seemed to be the most common, what gives

12_{II} (fig. 54), but 9_{I} (11_{II}) to 17_{I} (7_{II}) occurred and one pollen mother cell was found showing no reduction at all, the chromosomes dividing equational (fig. 55). On the whole, the chromosomes of this hybrid showed a great range of variation in their conjugation similarly as Cross 7. Elimination of chromosomes took place, just as also splitting of univalents, which may result in the formation of gametes with a higher number of chromosomes than any of the parents (see fig. 56 with 20 chromosomes).

The fertility of the hybrid was very poor, not more than 20—50 seeds per plant were obtained from hundreds of flowers, although these structurally were capable for selfpollination.

Cross 12: *V. tricolor*, $n = 13 \times$ *nana*, $n = 24$.

This hybrid is characterised by a certain amount of autosyndesis among the *nana*-chromosomes. Assuming that all *tricolor*-chromosomes conjugate with 13 *nana*-chromosomes, eleven *nana*-chromosomes are left without any mate, if no autosyndesis occurs. More than five univalents were never observed (fig. 61); the most common was three univalents, and even pollen mother cells with only one univalent were noted. If no trisomes or tetrasomes are formed this would give 16, 17 and 18 bivalents, respectively, the somatic chromosome number being 37. Trisomes or tetrasomes occur in almost all cells, but not more than one or two in each. Figs. 57—60 show heterotypic metaphases in side view, the three last ones are from a row in one and the same pollen mother cell; fig. 62 shows tetrasome, disome and monosome. The quality of the fixation did not allow the observation and counting of each conjugated unit, but the univalents were a rather safe indication of the autosyndesis, whether disomes or trisomes were formed. There is at least a striking difference between the behaviour of the *nana*-chromosomes in this hybrid and in Cross 18: *nana* $\times$ *arvensis* (figs. 86—89), in which only very few bivalents were formed.

Some elimination of chromosomes occurred and dwarf nuclei were formed, but the fertility of the hybrid was surprisingly good, as 1000—2000 seeds per plant were obtained.

Cross 13: *V. tricolor*, $n = 13 \times$ *rothomagensis*, $n = 17$.

As to chromosome number this cross is a homologue to Cross 9 (*tricolor* $\times$ *arvensis*), but the behaviour of chromosomes is different from this last one, because trisomes and tetrasomes together with a few monosomes are very common in the present hybrid (see figs. 63—64), not in the other one, and the fertility is also much less in

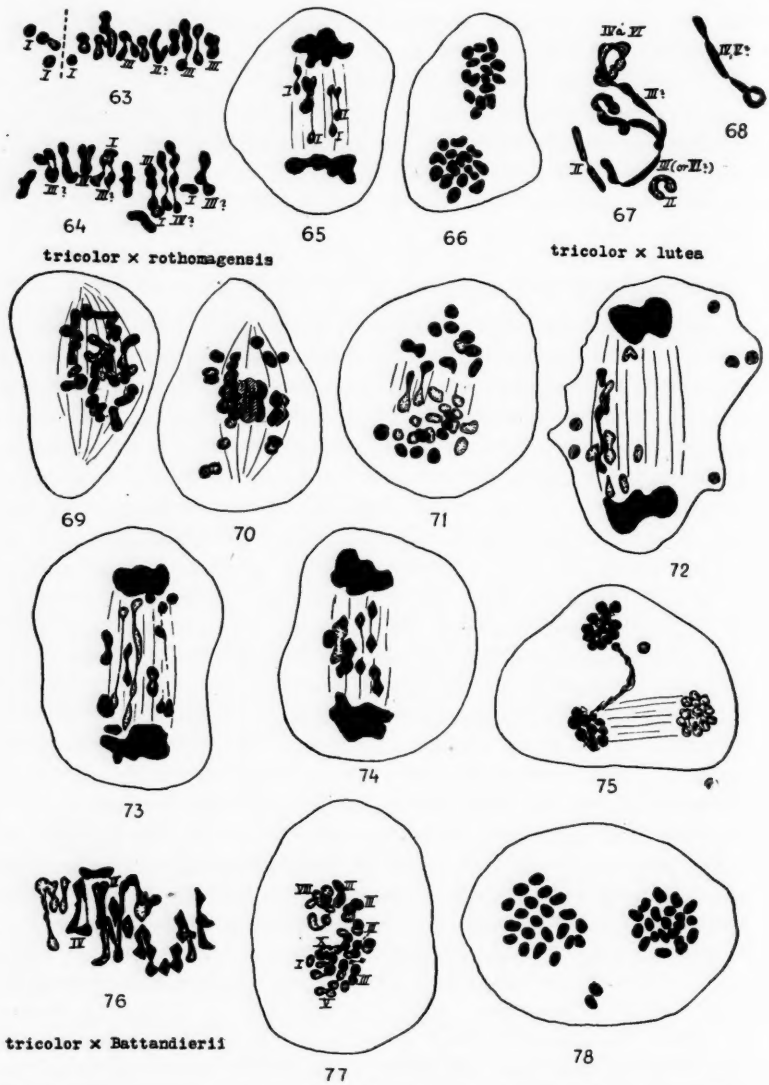
Cross 13 than in Cross 9; only 12—37 seeds per plant were obtained and but a small fraction of these germinated.

As in all *Viola* hybrids there were some variation regarding the amount of conjugated chromosomes in different cells (from two to six univalents were observed); some univalents divided in heterotypic anaphase (fig. 65). Most homotypic metaphases contain from 14 to 16 chromosomes (fig. 66); elimination of chromosomes and dwarf pollen were rather frequent.

Cross 14: V. tricolor, n = 13 × lutea, n = 24.

This cross is characterised by great irregularity in the progress of meiosis. The shortening of the individual chromosomes does not occur synchronously for all chromosomes of one and the same cell; some may be in the early diaphase (the so-called strepsinema) when others have shortened to the characteristic short shape of the late diaphase (fig. 67). As shown in this figure and in fig. 68 are some of the chromosomes connected to long, multivalent chains. The non-simultaneity and the chain formation often persists through heterotypic metaphase, but no regular zig-zag chains were observed, long and short chromosomes arrange themselves without any order in all directions between each others, sometimes connected to the most peculiar combinations; fig. 69 shows another type of heterotypic metaphase with short but irregularly arranged chromosomes. The fixation was done with NAWASHIN's fixative without previous application of CARNOY's fluid, and it did not allow the tracing of the individual bivalents through the heterotypic division. The univalents were most often belated enough for an observation during the heterotypic metaphase and anaphase; from 8 to 11 univalents were the most frequent (fig. 70), and there is a strong tendency for these to divide during anaphase as illustrated by the figures 72—74. The average chromosome number of the gametes increases by this process, fig. 71 shows 21—22 chromosomes at one of the poles of a heterotypic anaphase and in a previous paper (1927 b, fig. 72) a homotypic metaphase with abt. 23 chromosomes was reproduced. This is accordance with the fact that the cultivated pansies (*V. Wittrockiana* GAMS), which arose from this specific crossing in the years between 1830 and 1840 (WITTROCK 1896), have a chromosome number very near to that of the parent with the largest number, viz. abt. 24 (J. CLAUSEN 1927 b, figs. 73—77).

In some pollen mother cells were long chromosomes seen to persist to homotypic telophase connecting even the nuclear groups, which were



Figs. 63—78. Crosses 13—15. *63—66*: Cross 13; *63*: het. met. from two sections, abt. $3_{III} + 9_{II} + 3_{I}$; *64*: het. met., not complete, ca. $2_{IV} + 4_{III} + 2_{I}$; *65*: het. ana., univalents dividing; *66*: hom. met., n = ca. 16 and 14—15. — *67—75*: Cross 14; *67*: chromosomes from one cell in diaphase, unequally shortened, multivalent associations; *68*: polysome from diaphase; *69—70*: het. met., in *70* ca. 11_{I}; *71*: het. ana.,

separated at heterotypic anaphase, that is, the connection has persisted through all the phases lying between them. Fig. 75 shows such a chromatin connection, which reminds very much about the abnormal divisions described by GOODSPEED and AVERY (1930, text figures 8—17) from X-rayed *Nicotiana Tabacum*.

In spite of all irregularities, the chromosomes are in homotypic telophase generally found arranged in four groups, pentads are rare. An enumeration through one slide gave 29 tetrads and three pentads. In the pentads the fifth cell was always small. The individual pollen in the tetrads were often sligthly different as to size. The plasm contained plenty of nucleolar material. In spite of great efforts only 50—300 seeds were obtained per plant, and the germination was less than 10 per cent.

Cross 15: *V. tricolor*, $n = 13 \times$ *Battandierii*, $n = 26$—*30*.

As mentioned before, the chromosome number of the garden type of *V. Battandierii* is oscillating, and the gametes, therefore, do not all contain the same number of chromosomes. Anyhow, they contribute the largest number of chromosomes of any *Melanium* violet. The F_1-plants of Cross 15 had apparently not all exactly the same number of chromosomes. Contrary to expectation the univalents were few, less than six and usually not more than 3—4, not 13—17 as indicated by the difference in the chromosome number of the respective parents. This was due to a very extensive association of chromosomes into multivalent complexes (figs. 76—77) consisting of as many as up to ten chromosomes, as far as could be judged. This means a rather large degree of autosyndesis, but, in fact, even the non-hybrid *Battandierii* shows multivalent association of its chromosomes (fig. 15 *d*). The appearance of the heterotypic metaphase was very irregular and it was very difficult to interpret. Homotypic metaphases looked more ordinary, the nuclear plates contained most often 20 or 21 chromosomes (fig. 78) and the number oscillated between 23 and 17. Elimination of one to three chromosomes was very frequent, just as were pentads, hexads and heptads. The supernumerary pollen were generally minute

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the upper chromosomal group not complete, in the lower 21—22 chromosomes; *72—74*: het. ana.-telophase, univalents dividing, in *72* extranuclear nucleoli (hatched); *75*: hom. telophase, a chromosomal connection has persisted from het. met. — *76—78*: Cross 15: *76*: het. met., multivalent association; *77*: het. met. in polar view, abt. $1_X + 1_{VIII} + 1_V + 4_{III} + 2_{II} + 1_I + ?$; *78*: hom. met., 21 + ca. 20 + 2 chromosomes.

dwarfs, obviously representing eliminated single chromosomes or groups of chromosomes. The fertility was rather good and germination not poor, abt. 30 %.

Cross 16: *arvensis*, $n = 17 \times$ *rothomagensis*, $n = 17$.

These two species have one and the same chromosome number, but differ much as to their morphological characters. Meiosis of the hybrid was much more irregular than of the Crosses 4 and 8, in which the parent species also have the same number of chromosomes. From two to six univalents were noted (figs. 79—81), which means that one to three pairs of chromosomes failed to conjugate. In addition to the bivalents were one or two trivalents and occasionally a quadrivalent present. The cytological picture was not very clear and the chromosomes were often densely aggregated; this irregularity is probably connected with the fact that the nuclear membrane very often disappeared during the later prophase, before the diaphase. Univalents were seen dividing during heterotypic anaphase; detached single chromosomes, dwarf nuclei (rare), pentads and hexads were noted. Fertility was comparatively good, 250—450 seeds per plant were collected; the germination was no less than 30 per cent.

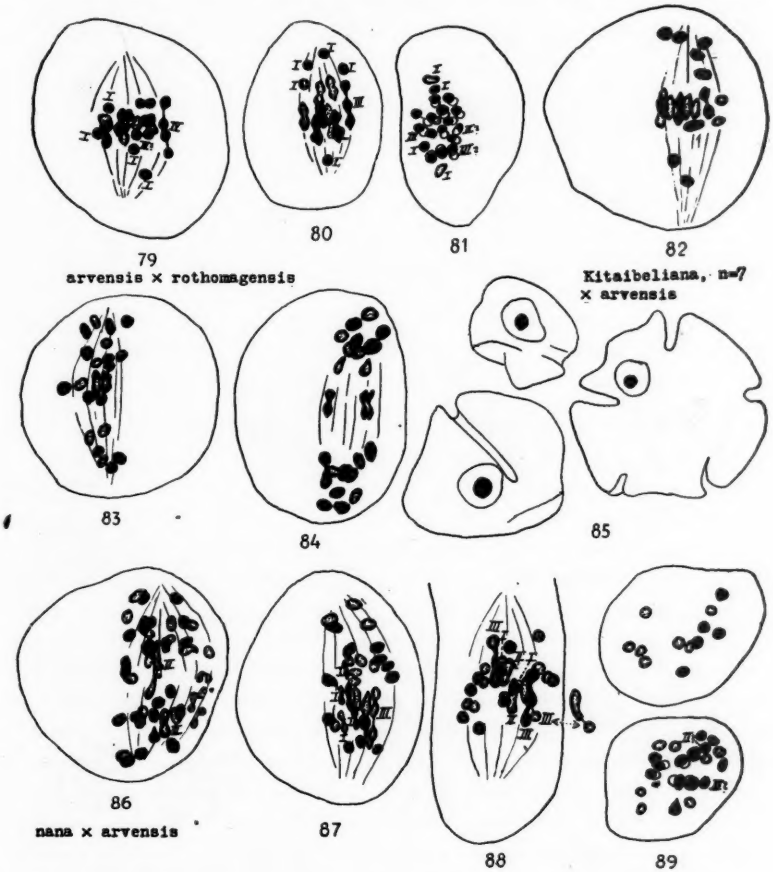
Cross 17: *V. Kitaibeliana*, $n = 7 \times$ *arvensis*, $n = 17$.

V. arvensis has ten chromosomes more than the *Kitaibeliana* type applied for the present cross giving ten univalents as minimum (fig. 82), but twelve univalents (corresponding to only six bivalents) were seen in most of the pollen mother cells. Occasionally also cells with fewer bivalents occurred (fig. 83: $2_{II} + 20_{I}$). Most univalents distributed themselves to the two poles, but detached chromosomes were noted. Some univalents divided during heterotypic anaphase (fig. 84) and, similarly, pentads and hexads were seen between more or less normal tetrads. The size of pollen was very variable (fig. 85), as it must be in a hybrid with so great difference between the parental numbers of chromosomes. The fertility of F_1 was considerably reduced as compared with the parents, but not so poor indeed, as 500—600 seeds were obtained per plant. The germination rate, on the other hand, did not amount more than 17—19 per cent.

Cross 18: *V. nana*, $n = 24 \times$ *arvensis*, $n = 17$.

This hybrid showed surprisingly few bivalents (more than six were never observed with certainty). The pollen mother cells were crowded with univalents (figs. 86—87). This is astonishing because Cross 12,

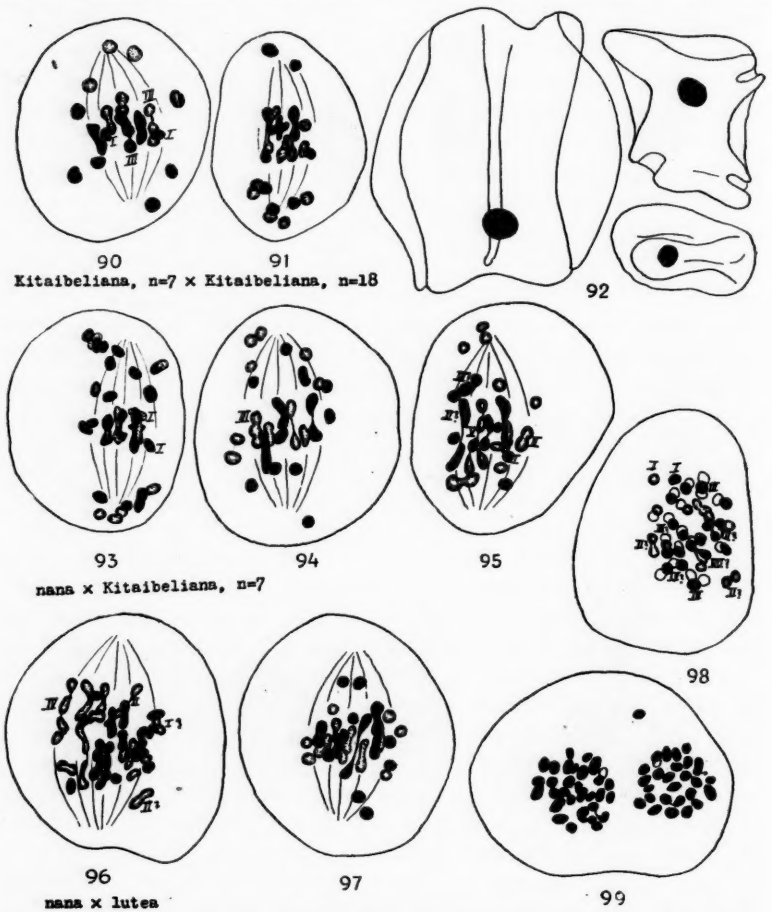
tricolor × *nana*, showed surprisingly *few* univalents. If the *nana*-chromosomes are capable for autosyndesis in Cross 12, why do they not



Figs. 79—89. Crosses 16—18. *79—81*: Cross 16, het. met.; *79—80* showing tetra-, tri- and monosomes; *81*: abt. $12_{II} + 2_{III} + 4_{I}$. — *82—85*: Cross 17; *82*: het. met., ca. 10_{I}; *83*: het. met., ca. $2_{II} + 20_{I}$; *84*: het. ana., ca. 10 + 3 (dividing) + 11 chromosomes; *85*: young pollen of different size (smaller magnification). — *86—89*: Cross 18, all het. met., *88—89* from embryosac mother cells; *86*: ca. $2_{I} + 37_{I}$; *87*: at least 21_{I}, probably 26—27, some ones dividing, one trisome; *88*: at least 18_{I} and 3_{III}; *89*: polar view in two sections, at least 26_{I}.

conjugate mutually, irrespective of any *arvensis*-chromosomes, in Cross 18? Embryosac mother cells of two plants were therefore inspected in

order to see, if the chromosomes showed better conjugation here than in the pollen mother cells, similar to what was observed in a pollen



Figs. 90—99: Crosses 19—21. *90—92*: Cross 19; *90*: het. met., ca. $2_{III} + 4_{II} + 11_{I}$; *91*: ca. $6_{II} + 13_{I}$; *92*: young pollen of different size, same enlargement. — *93—95*: Cross 20, het. met.; *93*: $5_{II} + 22_{I}$; *94*: ca. $1_{III} + 5_{II} + 18_{I}$; *95*: ca. $1_{V} + 1_{III} + 6_{II} + 11_{I}$. — *96—99*: Cross 21; *96—98*: het. met., in *96* one tetrasome and abt. 15 univalents, in *97* at least 15_{I}; *98*: (polar view) probably $2_{VII} + 1_{IV} + 3_{III} + 11_{II} + 2_{I}$; *99*: hom. met., in the nuclear plate to the right 26 chromosomes.

sterile type of *V. Orphanidis* (J. CLAUSEN 1930 b), but such was not the case (figs. 88—89).

Trisomes were rarely observed, and some univalents would split during heterotypic division. The fertility was rather good (250—800 seeds per plant) but considerably less than in Cross 12, *tricolor* × *nana*, although *arvensis* and *nana* unquestionably are morphologically more near related than are *tricolor* and *nana*.

Cross 19: *V. Kitaibeliana*, $n = 7$ × *Kitaibeliana*, $n = 18$.

Although these two parent types, morphologically considered, belong to one and the same species, the cytological irregularities are just as large as in species hybrids, and the sterility is more complete than in any other *Melanium* hybrid. There is a tendency to form six bivalents (leaving one of the seven chromosomes unconjugated) in addition to 13 univalents (figs. 90—91). One or two univalents may conjugate with bivalents giving trisomes. With so many univalents there is a possibility for a very uneven distribution of chromosomes during meiosis, and the pollen are actually very different in size (fig. 92).

Cross 20: *V. nana*, $n = 24$ × *Kitaibeliana*, $n = 7$.

This is the widest cross within the collective species of *V. Kitaibeliana*, a cross of an octoploid type with a hyperdiploid one, the difference between the chromosome numbers of the parents being 17. There is some variation in the conjugation of chromosomes in the hybrid. All the seven chromosomes from one parent do not ever find a mate among the 24 chromosomes of the other as some pollen mother cells did not show more than five bivalents (fig. 93 with $5_{II} + 22_{I} = 32$, the *nana*-gamete contributing to this F_1-plant must have carried 25 chromosomes instead of 24!). Trivalents were met with (figs. 94 and 95) and even a pentasome was observed (fig. 95); in this last case some sort of autosyndesis must have taken place. The pollen mother cells were well developed and stained well. The fertility of F_1 was not poor, 250—350 seeds were obtained per plant. Cross 20, as regards chromosome number and geographic habitat of parent types is a wider cross than Cross 19, but it shows less irregularity and less sterility.

Cross 21: *V. nana*, $n = 24$ × *lutea*, $n = 24$.

These two species have the same chromosome number and their areas are not remote from each others, but they are morphologically very different. They cross easily, 72 crossed seeds were obtained, giving 50 F_1-plants, which showed characters from both parents, although *nana* was prevalent regarding conspicuous characters as flower size and flower colour. The fertility was rather poor, from 50—300 seeds were

obtained from each of the richly branching plants. Pollen mother cells stained well, but the many chromosomes in no regular association troubled the disentanglement of the individual chromosomes and their associations. 15—18 univalents were observed in some cells (figs. 96—97) and irregular polysomic association occurred (fig. 96: a tetrasome, fig. 98: probably heptasomes, tetrasome and trisomes). Homotypic metaphases showed a little more or less than 24 chromosomes and occasionally detachment (fig. 99).

Cross 22: *lutea*, $n = 24 \times$ *Battandierii*, $n = 26$—*30* and

Cross 23: *Battandierii* $\times$ *calcarata*, $n = 20$.

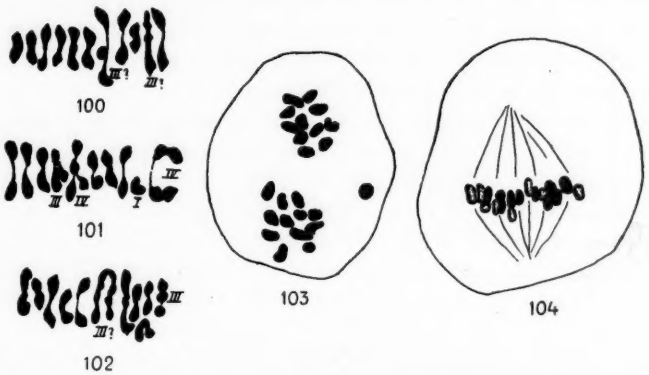
The two hybrids have not the primary cytological interest as the other ones mentioned due to the fact that the cultivated *Battandierii* type applied for the crossings had an oscillating number of chromosomes. *Cross 22* was fixed already in 1923 and owing to primitive technique then available (CARNOY fixation, DELAFIELD hæmatoxylin) it was impossible to get anything out of the heterotypic metaphase; the homotypic metaphases were fairly regular with about 24—26 chromosomes and occasional elimination of single chromosomes. The fertility was not good (50—100 seeds per plant) but the germination was fairly good.

Cross 23 also showed elimination of univalents and a varying number of chromosomes in the homotypic metaphase (24, 28, etc.) so as pentads with dwarf pollen. The fertility was more poor in Cross 23 than in Cross 22, but the present cross was, nevertheless, the only successful one with *V. calcarata* as a member. Only 18 F_2-plants were obtained, showing some segregation as to shape of leaves and flowers etc., but all were violet flowered.

Cross 24: (*V. tricolor*, $n = 13 \times$ *Orphanidis*, $n = 11$, *10*) $\times$ (*cornuta*, $n = 11 \times$ *elegantula*, $n = 10$).

As a matter of course, the two F_1-hybrids crossed together form gametes with a somewhat differing number of chromosomes, and the individual plants of the quadruple F_1, therefore, differ as to the number and the initial origin of their chromosomes so as to their morphological appearance and their fertility. It was surprising to see how regular the division was going on in hybrids with chromosomes from four species brought together. Figs. 100—102 illustrate heterotypic metaphase in V. 1205—4 with a total number of chromosomes of abt. 24, almost all conjugating, either as ordinary disomes or as tri- and tetra-

somes. This plant was somewhat fertile, giving 123 seeds and 22 F_2-plants from these. Figs. 103—104 illustrate another plant of Cross 24, the completely sterile V. 1205—2, also with a total chromosome number of 24 (somatic); fig. 104 shows two homotypic metaphases uniting into one very large group of chromosomes, which would give diploid pollen grains. The young pollen of this plant showed a tendency to disintegration, filling the pollen sac with a fibrillous mass similar to what was observed in pollensterile *V. Orphanidis* (J. CLAUSEN 1930 b).



Figs. 100—104. Cross 24 (quadruple hybrid). *100—102*: V. 1205—4, het. met., 2n = 24; *103—104*: V. 1205—2 (sterile), hom. met., 2n = 24, in *104* are both nuclear plates united to one.

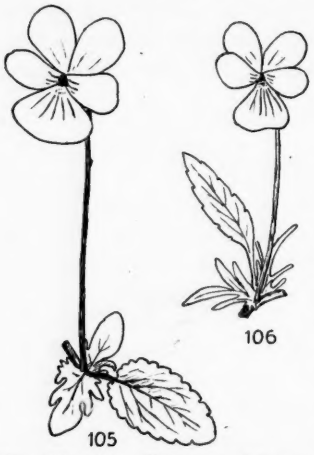
V. TYPES OF SEGREGATION IN F_2 AND LATER GENERATIONS.

Cross 1: *V. cornuta* (*Aa, violet*) × *elegantula* (*AA, reddish*).

A line drawing of F_1 is reproduced in fig. 105; it shows the characteristic intermediate stipules and leaves (compare figs. 2 and 4). Fig. 19 shows the characters of the flower (direction of lateral petals and extension of yellow eye) of the parents and of F_1.

Segregation of the *A*-gene (anthocyanous:*alba*) in F_2 and of the character yellow eye will be mentioned in chapter VI. For a number of other characters were segregations noted, but in these cases numerous gradations were observed covering the total range of variation with the characters of the two parents as the extremes, while classification was impossible. This holds true for the shape and diversion of the stipules, for direction, size and shape of petals, length of spur and partly also

for colour of flowers, except that true red flowered plants did not appear in F_2, but a large range of variation into more or less reddish types was noted (in addition to pure white). This may be connected with the fact that the *elegantula*-parent, V. 639—2, was not true red but of an intermediate colour and probably not constant for this colour, as the much more red types of later accessions were. Already in F_1 darker or lighter reddish types were observed, but all were more reddish than *cornuta*. Some plants of F_2 were of a perennial type and others looked just more like annuals.



Figs. 105—106. Flower and leaves of *cornuta* × *elegantula* F_1 (*105*) and of *tricolor* × *elegantula* F_1 (*106*).

In a backcross of one F_1-plant with a pure red and constant *elegantula* of another accession it was tried to classify the characters of the individuals into two classes, according to whether they belonged to the F_1-type of the character in question or they were similar to *elegantula*. F_1 was intermediate with respect to all the four characters tested. The enumeration is shown in table 6.

The 88 F_2-plants germinated from abt. 1000 seeds sown, and the 27 backcross-plants from abt. 140 seeds. Of 400 seeds collected from selfpollinations of four F_2-plants no one at all germinated. It is impossible to say what classes the non-viable combinations belonged to.

TABLE 6. *Classification of the individuals in the backcross V. elegantula* × (*elegantula* × *cornuta*).

Type	flower colour	flower shape	direction of lateral petals	shape of stipules
= *elegantula*	17 (red)	14	9 (upwards turned)	18
= F_1	10 (reddish violet)	13	18 (horisontal)	9
total	27	27	27	27

No *alba*, all with yellow eye as expected.

This cross is of interest, because it shows that also characters distinguishing larger taxonomical units in this section segregate and

must depend upon the interaction of genes, apparently not very few. Cross 5, *V. Orphanidis* × *cornuta alba*, showed a similar segregation as to shape and direction of petals so as to presence or absence and extension of yellow eye.

Cross 6: V. tricolor alba-yellow × *elegantula, red.*
($a_1a_1a_2a_2RRLL$ × A_1A_1 . . *rrll.*)

The 30 F_1-plants germinated from 40 seeds and varied a little (the *elegantula* parent did not belong to any pure line). The F_1-type of flower, leaf and stipules is shown on fig. 106 (compare the parental species, figs. 2 and 6, but the *tricolor* parent had smaller flowers due to the absence of all *A*-genes). The shape of leaves and stipules was greatly influenced by *V. tricolor*. Young flowers of F_1 were whitish, changing to paler or darker violet (action of one gene for yellow, *L*, and the *R*-gene, both from *tricolor*). Some minor variation as to intensity of colour was noted. A number of the F_1-plants belonged to a sterile bushtype, which did not flower, neither the first, the second or the third year. Backcrossing of F_1 to one of the two parents gave considerably more and considerably better seeds than the selfpollination (see table 7).

TABLE 7. *Average number of seeds per capsule of V. 1104, Cross 6, F_1.*

Plant no.	1	2	3	4	total of seeds	plants germinated
self pollination	1	0,8	1,4	2,6	50	6 (from 32 seeds)
F_1 × *tricolor alba*-yellow	3,3	3,9	—	—	181	64
F_1 × *elegantula*.....	—	—	10,8	5,6	181	96

The segregation as to the *A*-gene in F_2 and backcrosses is mentioned in chapter VI. A considerable number of plants of both of the backcrosses never attained flowering; they belonged to a peculiar minute and tufted dwarfish type, many of them much more extreme than the corresponding types in F_1. The numbers of this type were the following:

	normal	sterile dwarfs	total
F_1 × *tricolor alba*-yellow	34	24	58
F_1 × *elegantula*	67	23	90

As to flower colour *elegantula* seems to possess some intensifiers and extension genes obscurating the segregation. By the action of

these, *Ll* may become rather bright yellow, not yellowish white changing to pale violet as usual. Table 8 gives the classification of the different categories of flower colour.

TABLE 8. *Segregation for flower colour in Cross 6, tricolor × elegantula F_2 and back crosses.*

Antho-cyanin	flower colour	formula	F_2	F_1 × *tricolor* *alba*-yellow *AaRrLl* × *aaRRLL*	F_1 × *elegantula* *AaRrLl* × *AArrll*
with	violet.....	*ARll*	—	10 (violet + *pallida*)	24 dark violet
	rose	*Arrll*	1		32 dark rose
	pallida	*A. Ll*	3		11 yellowish
	yellow	*A. LL*	1	10	—
without	*alba*	*aa.* *Ll*	—	9	—
		aa. *ll*			
	alba-yellow...	*aa. LL*	—	5	—
		total	5	34	67

TABLE 9. *Segregation of supposed RrLl-plants of Cross 6.*

Flower colour	F_2	F_3		total	calcu-lated	ratio
	V. 1218	V. 1345	V. 1348			
ARll violet	—	7	28	35	29,8	3
Arrll red	1	1	6	8	9,9	1
A.Ll pallida	3	8	79	90	79,5	8
A.LL yellow.....	1	7	18	26	39,8	4
total	5	23	131	159	159,0	16

Four of the five F_2-plants were selfed for an F_3. Two of them segregated types similar to F_2 (see V. 1345 and 1348, table 9). A pale rose plant gave two pale and three darker rose.

A fourth F_2-plant, yellowish flowered with upper petals rose, (classified as »*pallida*»), gave 20 F_3-plants; 8 of them were yellow with upper petals rose and 12 were of an entirely new type with all petals yellow. Fig. 107 shows flowers of these types with their parent species. *tricolor alba*-yellow and *elegantula* in the upper row and two flowers of each type in the lower row, the intense yellow type to the right; this has larger flowers than the type with upper petals rose. The intense

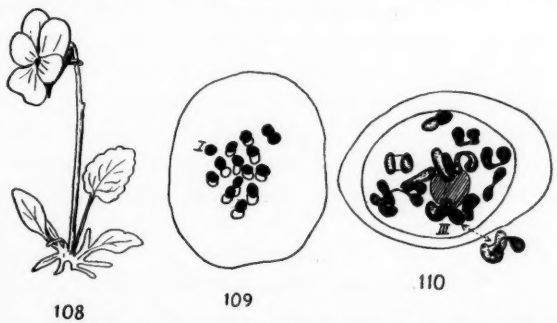
yellow type was constant, two F_4-sowings of it gave 19 and 43 intense yellow, respectively. A plant with upper petals rose segregated 18

Fig. 107. Upper row: *tricolor alba*-yellow (left) and *elegantula* (right); lover row: flowers of F_4-types from this cross; left: yellow-rose, right: intense yellow on all petals (*V. phæno-elegantula*).

yellow-rose : 11 intense yellow. The growth habit and entire vegetative appearance of this new type, which may be referred to as

Viola phæno-elegantula,

(after its bright and very showy flowers) is that of a *Viola elegantula,* but the stem nodes are shorter; it is very cespitose and each plant forms



Figs. 108—110. *Viola phæno-elegantula. 108*: Flower, leaf and stipules; *109*: F_3 of the original cross, het. metaphase, 13_{II} (one horisontal) + 1_I; *110*: F_4, diaphase, $11_{II} + 1_{III}$.

a low, dense tuft with numerous, bright yellow flowers raised above the decumbent stems. Fig. 108 is reproduced from a line drawing of leaves and flower; the spur is thin and straight, but somewhat shorter

than of elegantula; the flowers are broader than of any of the parental species. From the *tricolor*-parent the *L*-gene for yellow flowers has been transferred, while *elegantula* probably has contributed the intensifying gene or genes. Although *V. phæno-elegantula* morphologically spoken has been built up mainly of characters from *elegantula*, it presents several new traits in its structure, in first line its flower colour, and it proves that genes from one species may be added to a bloc of genes from another and still form a viable combination. *V. phæno-elegantula* is namely very fertile and gives large capsules with many seeds. Although morphologically of a fairly constant type, it is not in chromosomal equilibrium as yet. Fig. 109 shows heterotypic metaphase of F_3 of it; the total chromosome number of this plant was 27, most often conjugating $13_{II} + 1_I$, but one of the bivalents is usually placed horisontal outside the other bivalents together with the univalent one (compare F_1 of Cross 1). Fig. 110 shows diaphase of an F_4-plant, selfed offspring of the F_3-plant represented in fig. 109. The total chromosome number is 25 and a number of diaphases show 11 bivalents plus one trisome, but occasionally may also three univalents occur. Dwarf pollen are frequent in tetrads of this plant, and the type may be supposed to stop at the chromosome number of 12_{II}.

Cross 7: V. tricolor alba-yellow × *Orphanidis, violet.*
($a_1a_1a_2a_2LLvv$ × $A_1A_1..llVv$.)

Although the *Orphanidis* type applied for crossing had one univalent chromosome, it was a constant type, never showing any conspicuous morphological segregation. Hundreds of plants from self-pollination were always violet flowered (J. CLAUSEN 1930 b), never yellow. F_1 of the present cross showed a conspicuous and surprising segregation as one half of them were dark violet (not pale violet as expected from the presence of one *L*); another half had intense yellow lower petal with the upper petals of young flowers whitish or pale mauve, later on violet, while the lower petal remained yellow. The actual numbers of F_1-plants were 46 of the violet type as compared with 56 of the yellow one, being a total of 102 plants. As the *tricolor* parent was known to be homozygotic, this segregation suggested that the *Orphanidis* parent, V. 917—1, was heterozygotic for a gene, whose segregation was concealed in the pure *Orphanidis*, because it needed some activation of a gene from *tricolor*. But it seems more natural to admit two types of genes for violet colour: one of these, probably

identical with the *A*-genes, is the usual basal violet colour of all *Melanium* species. It is hypostatic, may be concealed by *LL* and *WW* and changed to red in the absence of *R* and to more or less dark *velutina* by the absence of genes of the *M*-series. The other gene for violet colour, *V*, is supposed to be dominant and epistatic over yellow, *LL*, but it needs the presence of one *A*. It is a condition in analogy with what WINGE (1920) assumed for the colour of horses, namely (1) a dominant gene for red, partly epistatic to black, and (2) a recessive red, the red basal colour of the horse, hypostatic to black.

If *Orphanidis* is *Vvll*, it cannot segregate yellow because it has no *L*-gene, and the *vvll*-plants segregated are violet due to the presence of *A*-genes. But if this *Orphanidis* is crossed with *tricolor alba*-yellow, *vvLL*, half of the plants get *VvLl* and are violet by the epistatic action of the *V*-gene; half of them get *vvLl* and are yellow, not pale violet as usual, due to some dominance of an intensified *L*-gene and the absence of the *V*-gene.

111

Fig. 111. Flower, leaf and stipules of Cross 7, *tricolor* × *Orphanidis*.

The F_1-plants had stipules intermediate between the two parents (compare fig. 111 with figs. 5 and 3), the leaves, flowers and direction of stems (erect) resembled more *tricolor*; the herbage was minutely puberulent of very short but dense hairs. It was extremely sterile, and only by a careful self-pollination and troublesome back crossing were seeds enough obtained for segregations. The fertility was considerably better in the back cross. Table 10 gives the numbers of seeds obtained per capsule and the germination rate.

In F_2 (see the diagram, fig. 112) the violet F_1-plants segregated yellow ones and the yellow F_1-plants segregated violet ones. This sounds like a paradox but is nothing but what should be expected from the formulæ suggested. Likewise violet F_1 backcrossed with *tricolor alba*-yellow segregated violet and yellow, while yellow F_1 fertilised with the *tricolor* parent gave nothing but yellow plants.

F_3 corresponded to expectation as regards types except that some yellow F_2-plants coming from violet F_1 should again segregate violet in F_3; four plants gave constant yellow offspring, although $^2/_3$ of them should be *vvLl* and segregate violet, *vvll*. With the small number tried

there is a chance for them all being *LL*, especially if differential viability or elimination of chromosomes plays in with. The segregation of the basal gene *A* for anthocyanous colour is mentioned in chapter VI.

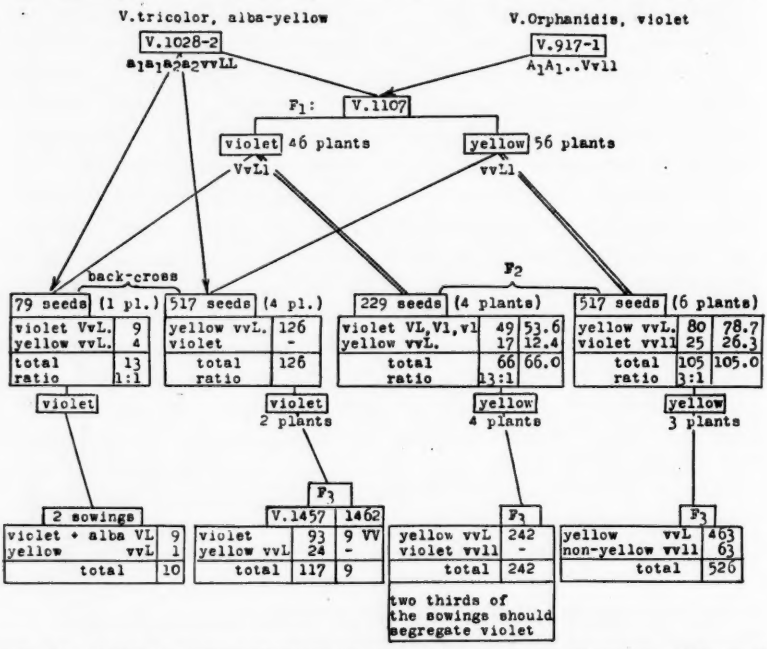


Fig. 112. Cross 7, diagram showing the segregation of flower colouring. (The ratio in F_2 from violet F_1 plants erroneously given as 13 : 1, read: 13 : 3.)

TABLE 10. *Average number of seeds per capsule of V. 1107, Cross 7, F_1.*

Plant no.	1	2	3	4	5	8	seeds total	seeds sown	plants germinated
selfpollination.....	2,4	3,8	2,6	1,5	3,9	5,5	766 (11 plants)	746	178
$F_1 \times$ *tricolor alba*-yellow	4,4	8,1	6,9	7,7	6,0	3,3	641 (8 plants)	596	142

It was surprising that of the 171 F_2-plants (see diagram, fig. 112) no one was of *Orphanidis*-type. This applies to flower shape, direction of stem (F_2 mainly erect), hairiness and shape of stipules. F_2 was of a glabrous appearance, no ones were hirsute as *Orphanidis*, although

several were puberulent; stipules of many plants showed influence from *Orphanidis*, but there were no true *Orphanidis*. No sufficiently reasonable explanation of this phenomenon can be offered. The crossing was not done reciprocal, and the F_2 plants resembled their *tricolor* grandmother as to type but showed segregation for many characters. The possibility is not excluded that it may be due to plasmatic influence from *tricolor*. Reciprocal crossing will decide this question. But there are other possibilities also, for instance that the two extra *tricolor* chromosomes exercise an essential influence upon the determination of type (most plants seem to have the extra chromosomes) or that the *Orphanidis* type is very recessive.

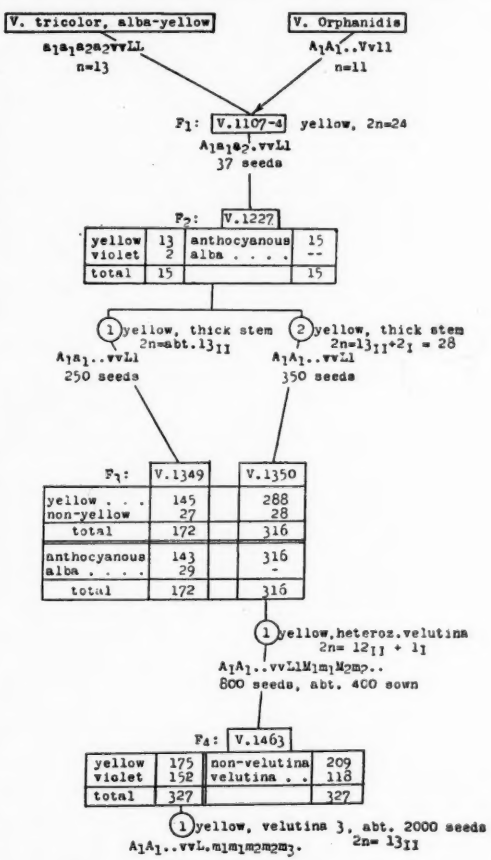
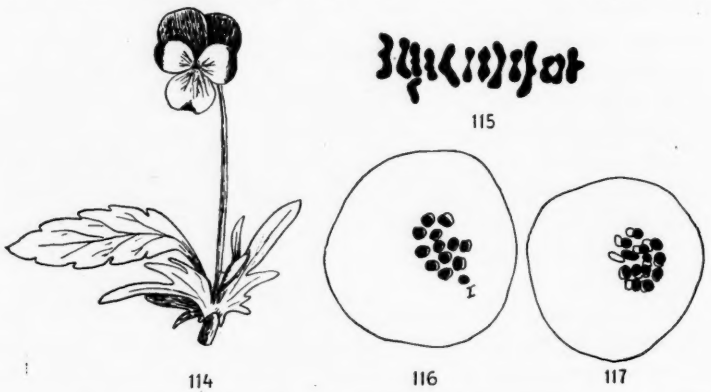


Fig. 113. Pedigree of *Viola crassicaulis*.

Some of the F_2-plants were noted to have very thick stems and were suspected of being tetraploid or at least with increased chromosome number. It will be remembered that some F_1-pollen mother cells showed almost no conjugation, but this was not connected with much splitting of univalents. Two F_2-plants of the thick-stemmed type were selfed and gave many seeds (compare the pedigree on fig. 113); the seeds germinated comparatively well, but none of the two plants were tetraploid; one of them, V. 1227—1, had about 13 bivalent chromosomes, another one, V. 1227—2, had 2n = 28, some-

times forming 14_{II} (fig. 115), sometimes $13_{II} + 2_{I}$. The offspring of these two plants (V. 1349 and 1350 in fig. 113) were mainly of the thick-stemmed type, being an instance of *gigas* growth without tetraploidy. In V. 1350 appeared two unexpected *velutina* aberrants with upper petals faint velvety, one of them, V. 1350—1, also had a faint velvet spot on the lower petal. This plant was extremely thick-stemmed and very fertile, giving abt. 800 seeds; the chromosome number was 25 ($12_{II} + 1_{I}$, fig. 116). About half of these seeds were sown and gave a total of 327 plants (V. 1463), a very vigorous culture. The flower colour ranged from pure yellow (intense or more pale) to dark



Figs. 114—117. *Viola crassicaulis*. *114*: Flower, leaf and stipules; *115*: V. 1227—2 (= F_2), het. met., 14_{II} (probably one IV), other plates of this plant showed 2_{I}; *116*: F_3, V. 1350—1, het. met., $12_{II} + 1_{I}$; *117*: F_4, V. 1463—1, het. met., 13_{II}.

violet. A large number of the plants had more or less velvety petals and at least one of them was of the recessive *velutina* 3 type with a velvet border on the lateral petal and a triangular velvet blotch on the lower petal. This plant, V. 1463—1, was selfed and gave abt. 2000 seeds; it had 13 bivalent chromosomes (fig. 117). Fig. 118 shows in the upper row the two parental species and in the lower row some flower types of their descendant, V. 1463, the flower most to the right being of V. 1463—1.

For convenience sake this line of population is named

Viola crassicaulis,

referring to its very thick and vigorous stems. Fig. 114 is reproduced from a line drawing of this type; the leaves are correlated with the stems and are large and rather thick. The stipules remind somewhat

about those of *Orphanidis*. The flowers are rather large but as compared with the leaves they are small; the petals are of a very broad shape, reminding about the cultivated pansies.

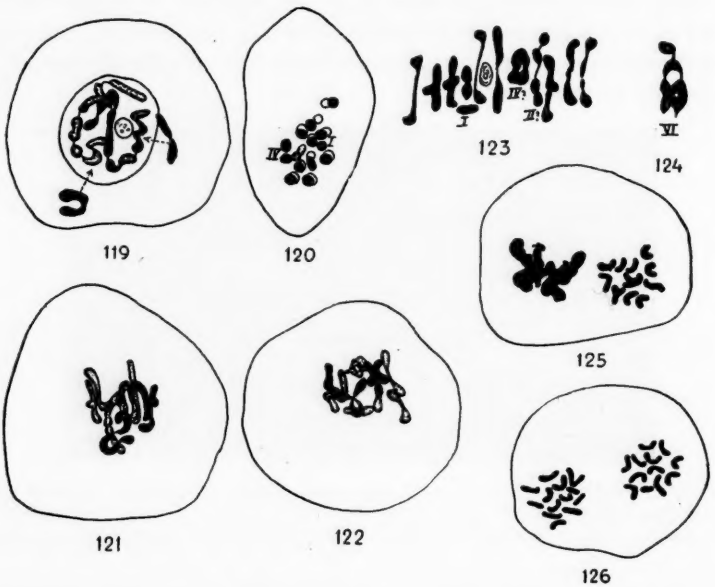
There is no doubt that this vigorous type of a fertility as large as in *arvensis* would have a chance in nature. Curiously enough it has stopped at the chromosome number of 13, that of its *tricolor* parent. If a type like this was found somewhere on the Balcan Peninsula, it would no doubt be described as a new species; a number of the types from this area in the neighbourhood of *V. Orphanidis*, *elegantula* and the Balcan *alpestris* type, *V. macedonica* BOISS. et HELDR., probably arose in a similar way.

Fig. 118. Upper row, left: *tricolor alba*-yellow; right: *Orphanidis*. Lower row: flower types of *V. crassicaulis*, V. 1463 (F_4); different degrees of velvety violet, the left one pure yellow, the right one is V. 1463—1 (*velutina* 3 and probably constant).

Another point of interest in Cross 7 are the changes which take place with the chromosomes during later generations of some of its types. There is no doubt that a more close following up of the behaviour of chromosomes in offspring of known hybrids would clear up many problems and shake some theories at their base.

F_1 of Cross 7 did not show much multivalent association of chromosomes (figs. 40—44), but some of the later generations show much more. Probably the conditions in hybrids favour a segmental interchange between non-homologous chromosomes. Figs. 119—126 illustrate meiosis in two violet flowered F_3-plants, one of V. 1457, another of V. 1462 (see the diagram, fig. 112). In diaphase of the first one, fig. 119, the chromosomes were connected in two long chains with some 10—11 in each and two free pairs; in the chains there appeared

to be a paired arrangement, but the pairs were connected end to end with short strands; (in one of the chains two pairs are seen parallely connected). This arrangement in chains was not kept so strictly through all heterotypic divisions (fig. 120 shows a fairly regular heterotypic metaphase with only one chain of four, an uneven pair and one univalent), but most of them were irregular and impossible to disentangle; the somatic chromosome number of this plant was 25.



Figs. 119—126. Unexpected chromosomal types and associations in F_3 of Cross 7, *119—120* are from one plant, *121—126* from another one, and they came from different F_2-parents. *119*: Chains of chromosomes in diaphase; *120*: a comparatively regular het. meta-anaphase in polar view, $1_{IV} + 10_{II} + 1_I$. *121—122*: Early het. metaphases, multivalent association of chromosomes in irregular chains; *123*: a less intricate het. met., but chromosomes very long, not as ordinary *Viola*-chromosomes, probably $1_{IV} + 10_{II} + 1_I$ (and one nucleolus); *124*: a peculiar hexasomic association; *125*: hom. metaphase, $n = 12$; *126*: hom. anaphase, $n = 13$, 12; long, bent chromosomes as in *Polemonium* and in many *Compositæ*.

In the plant of V. 1462 (figs. 121—126) a change as to meiotic chromosome shape had taken place and the chromosomes were also in this one connected to long, somewhat irregular chains even in early heterotypic metaphase (figs. 121—122); the arrangement was never in regular zig-zag chains or rings as in *Oenothera*. It is surprising, still,

how the chromosomes are able to disentangle themselves during meiosis in such a manner that they become distributed into two almost equal groups. As far as could be seen the diploid chromosome number was 25 (fig. 126), and the countings in homotypic metaphase ran as follows:

n =	11	12	13	14
number of nuclear plates	1	4	3	2, total: 10.

Most heterotypic metaphases maintained the multivalent association between the chromosomes, and the associations could appear in peculiar shapes as the hexasome seen in fig. 124 or in still more complicated connections. Some of them, indeed, could be fairly regular as that shown in fig. 123 with one quadrivalent, 10 bivalents and one bivalent; two of the pairs here resemble tetrasomes, but the total chromosome number of the plant indicates that at least one of them was only a disome.

The bivalent chromosomes were changed to long rods, and one or two of them could assume the shape of crosses (fig. 123); in the homotypic division they were bent in the shape of V's similar to somatic chromosomes. This shape of meiotic chromosomes is extraordinary for Violets, but other large plant groups exist, in which it is the normal one, for instance *Crepis* and many other *Compositæ*. It was also noted in *Polemonium* (J. CLAUSEN 1931 a). MANN LESLEY and FROST (1927) described a *Matthiola*, which deviated from normal *Matthiola* in a similar manner by having long chromosomes. By courteousy of Dr. FROST the writer had the opportunity of having this type in culture and was able to verify the statements of the two authors as to chromosome shape; in heterotypic and homotypic divisions it corresponds fully to the type of the long chromosomes in the genera mentioned. MANN LESLEY and FROST found the long chromosome type to be recessive, probably depending upon only one gene in their case. It is interesting to note that such shape or such conduct of chromosomes, which seemingly is of an essential nature as characterising large taxonomic units, may arise suddenly after crossing in part of the offspring, not in all of it and that it may probably arise by a single mutation also. This does not speak against the taxonomic value of such characters, it only shows that they may arise sporadic, when a proper combination of genes is realised, and it is a hint about the way, in which such differences arose.

The polysomic arrangement of chromosomes of the two plants mentioned resembles very much the illustrations recently published by

KATTERMANN (1931) of multivalent association between chromosomes of *Anthoxanthum odoratum* and *Bromus erectus*, regarded as good species.

Cross 8: V. tricolor × *alpestris and reciprocal.*
(ddll × *DDLL.)*

The yellow flower colour of the *alpestris* types applied is not so intense as in yellow flowered *tricolor*, apparently due to the action of some faintly bleaching gene. F_1 of violet *tricolor* × yellow *alpestris* is therefore violet, not whitish violet as F_1 of violet *tricolor* × yellow

Fig. 127. Cross 8 C, *tricolor alba* × *alpestris velutina*, flowers. Upper row from left: *alpestris* and *tricolor alba*; under them F_2-types. Middle row from left: violet, yellow, yellow *velutina* 1, and the rare, maculate, *alpestris* parental type; lower row: two flowers with local mutations to *velutina* in upper petals, one of them also petaloid; dilute mauve and *alba*.

tricolor (compare the flower colours on plate I, J. CLAUSEN 1926). Correspondingly, yellow of the *alpestris* cross has to be classified as a recessive, although in yellow plants the violet no doubt is still present, but concealed by the action of homozygous yellow. The violet will appear at spring time and when local mutations occur, as in the two flowers to the left in lower row of fig. 127.

Taken in this way the classification as to yellow lower petal was reliable. Table 11 shows this segregation for three different crosses

(A, B and C). The yellow from *alpestris* yellow-*velutina* (= *polychroma*) seems to give a more vital type (Crosses B and C) than the yellow from *alpestris typica* (Cross A), because in Crosses B and C

TABLE 11. *Segregation of flower colour in F_2 of tricolor × alpestris (all three crosses reciprocal): ll × LL.*

Flower colour	Cross 8 A: *tricolor* violet × *alpestris* yellow		Cross 8 B: *tricolor* violet × *alpestris polychroma*		Cross 8 C: *tricolor alba* × *alpestris polychroma*	
	observed	calculated	observed	calculated	observed	calculated
light to dark violet, $ll + Ll$	512	503,3	819	885,7	507	600,7
yellow, LL ...	159	167,7	362	295,3	294	200,3
total	671	671,0	1181	1181,0	801	801,0
		non velvety	1120		723	
		velvety	61		78	
		total	1181		801	

yellow is considerably in excess of its calculated proportion. Among those classified as violet there were a large scale of lighter and darker shades.

The velvety blotch on the two upper petals of *alpestris polychroma* is a peculiar character in its inheritance, but it is subject to phenotypical modification. It was apparent that very few velvety plants occurred in F_2, and most of them were only faintly velvety, very few had the typical blotch on the upper petals, rarely they would be total *velutina* on the two upper petals; fig. 127 shows flowers of parents and F_2 of Cross 8 C.

Cross C showed that *alpestris* has two polymeric (multiple) genes for anthocyanin similar to *tricolor*, but in addition it contains the gene *D* for dilute mauve similar to *arvensis* as will be seen from the enumeration below:

		observed		calculated	
anthocyanous.....	*A*	801			
dilute mauve	*aaD* . { *ll* / *Ll*	18		23,6	
alba.....	*aadd* { *ll* / *Ll*	12	42	7,9	42,0
alba-yellow	*aa* . . *LL*	12		10,5	

The crosses stress the position of *V. alpestris* as being specific different from *tricolor*, partly by the minor irregularities in meiosis of F_1 and partly by aberrant types occurring in F_2, especially the petaloid types (see fig. 127 and table 17 in Chapter VI). But apparently it stands just on the verge of being specific different and is therefore an interesting type illustrating that the conception of species must be conventional in some cases.

Taxonomists have sometimes thought that *alpestris* is more related to *arvensis* than *tricolor* is, and the genetical analysis verifies this assumption. In common with *arvensis* it has the genes *L* for yellow and *D* for dilute mauve, which are absent from typical *tricolor*, and it segregates petaloid and *velutina* aberrants, when crossed with *tricolor*, just as *arvensis* does, but it gives no semisterile types as the cross *tricolor* × *arvenis*.

Cross 9: V. tricolor × *arvensis.*

This cross has been dealt with previously (J. CLAUSEN 1926), but there are some additions to be made with regard to the character *dilute mauve* and to later generations of some constant types illustrating the origin of new species.

TABLE 12. *Inheritance of dilute mauve (D), extracted from arvensis, introduced into tricolor and crossed with different tricolor-varieties (all reciprocal).*

Flower colour, anthocyanin in stems		formula	dilute mauve × rose-*velutina* [$a_1a_1a_2a_2DDRR(ll) \times A_1A_1A_2A_2ddrr(ll)$]		dilute mauve × *alba* ($aaDD \times aadd$)		dilute mauve × *alba*-yellow ($aaDDll \times aaddLL$)	
			observed	calculated	observed	calculated	observed	calculated
anthocyanous		A...	713	705,0	—	—	—	—
green *aa*	dilute mauve	*D . ll*	28 [1]	35,3	391	390,7	54	45,2
	mauve-yellow	*D . Ll*	—	—	—	—	76	90,4
	alba	*ddLl*	11	11,7	130	130,3	50	45,2
		ddll						
	yellow	*.. LL*	—	—	—	—	61	60,2
		total	752	752,0	521	521,0	241	241,0
		ratio		60 : 3 : 1		3 : 1		3 : 6 : 3 : 4

[1] 8 of these dilute rose.

The gene, *D*, for dilute mauve was first proved to be present in *Viola arvensis* as shown by the Cross *V. tricolor alba* × *arvensis* (J. CLAUSEN 1926, Cross XIV, p. 107). Via a backcross of F_1 to *tricolor alba* and selfing through two successive generations a type was selected, which was a true *tricolor* as to morphological characters and with 13 bivalent chromosomes as *tricolor*, but with the *D*-gene introduced from *arvensis*. This type was crossed with some *tricolor* varieties, before it was homozygous for the *D*-gene, and F_1-plants with *D* were selected for F_2. Table 13 shows these segregations, which are not too far from expectation. Dilute mauve in a single dose is seen to be epistatic to

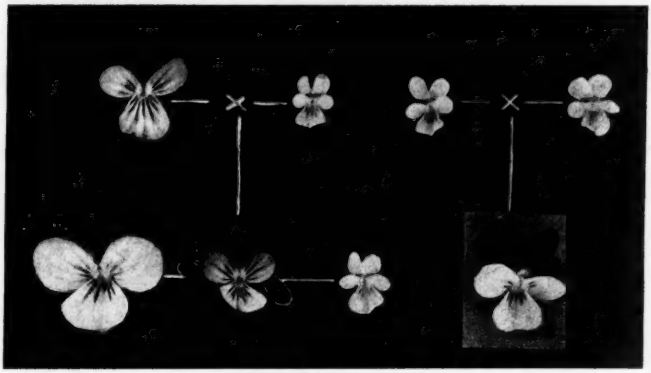


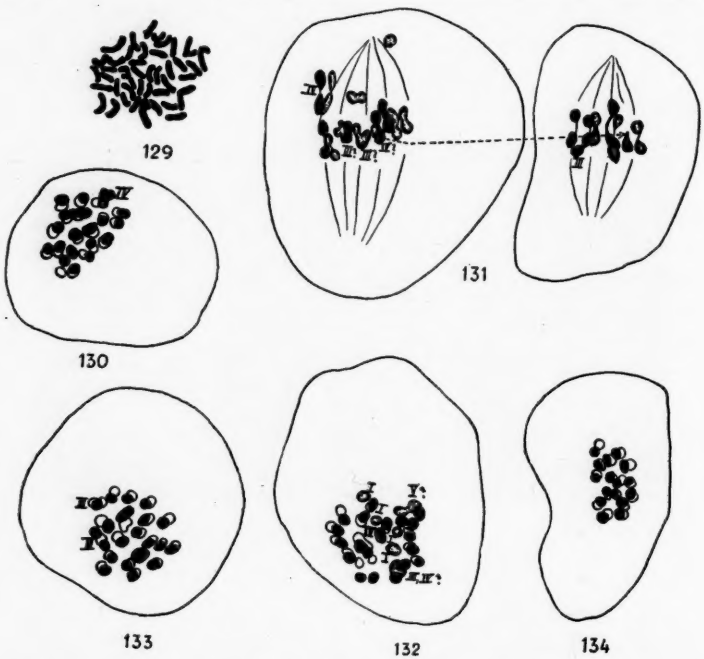
Fig. 128. New and constant types produced by crossing of *tricolor* and *arvensis*. Left part: *tricolor typica* and *arvensis* Line 52 with three types, namely from left: *V. hyperchromatica* ($n = 21$—23), *V. petaloidea* (14_{II}) and a constant segregated *arvensis* (16_{II}). Right part: *arvensis*, Line 52 and *arvensis*, Line C, both with 17 bivalents; under them their aberrant, *V. velutina*, $n = 16$ (loss of one chromosome).

heterozygous yellow, *Ll*, but homozygous yellow is epistatic to dilute mauve, as well *DD* as *Dd*.

Of the constant types described in the 1926-paper were some few selected to be propagated generation after generation in order to try them for constancy and find out the chromosome number at which they would stop, if changes took place. Flowers of these types are shown in fig. 128 together with their parent types (see explanation under the figure).

Viola hyperchromatica is described on pp. 126—130 of the cited paper. It arose from a partial duplication of the chromosomes in meiosis of a single F_1-plant, which formed gametes with chromosome number ranging from abt. 15 to 25, giving plants with a somatic

chromosome number of abt. 39 to 46. Under ordinary conditions are 11 to 13 *arvensis*-chromosomes capable of conjugation with the same number of *tricolor*-chromosomes; it is therefore evident that a duplication of chromosomes as in *hyperchromatica* may create some possibility for the formation of tetrasomes and trisomes. Such are actually found and most nuclei, indeed, contain tetrasomes and trisomes and con-



Figs. 129—134. Chromosomes of *V. hyperchromatica* (*129—133*) and *V. velutina* (*134*). *129—131*: F_7 of the original cross, *129* shows a somatic chromosome plate, $2n = 42$; *130*: 18_{II} + at least one IV; *131*: het. met., abt. $2_{IV} + 3_{III} + 12_{II} + 1_I$. *132*: F_8, het. met., 2n between 40 and 43, a pentasome, tetrasomes, monosomes and probably also trisomes; *133*: F_9, het. met., $2_{III} + 18_{II} = 42$. *134*: *V. velutina*, het. met., 16_{II}, not entirely regular in their arrangement (F_5).

sequently also monosomes. The chromosome number varies a little from plant to plant, although it generally lies about $2n = 42$. But the morphological type has been maintained unafflicted by the slight changes in chromosome complement. Fig. 128 shows its appearance in fall, when it attains its fullest development as compared with *tricolor*. Figs. 129—131 show chromosome complement of an F_7-plant, 132 of

F_8 and 133 is of F_9 as calculated from the original crossing of *tricolor* × *arvensis*. *V. hyperchromatica* in respect of chromosome irregularity is not different from »good» species growing in nature, as shown in another paper (J. CLAUSEN 1931 b). It is fairly fertile and gives abt. 300—400 seeds per plant.

The name *Viola petaloidea* will be used for the constant 14-chromosome type with coloured sepals (type V. 616, J. CLAUSEN 1926, p. 100). It was obtained as a constant large flowering type; especially during spring and fall are the two lateral sepals very large and coloured as in fig. 128. The chromosome divisions are regular. It is the only *Viola* species hitherto found with 14 chromosomes and with petaloid sepals. One plant of F_7 gave 800 seeds.

The segregated *constant arvensis-type* (l. c., V. 607, V. 740, p. 99) shown as number three from the left in the lower row of fig. 128 attained constancy with 16 bivalent chromosomes in F_5. It has been cultivated to F_8. Morphologically it was constant before this. From *tricolor* it received the character of dark spot in front of the style, and it is extremely fertile.

To the right of last named (fig. 128) is shown another 16-chromosome type but distinctly different from it. For this type the name *Viola velutina* will be applied. It arose from a crossing between the two *arvensis*-types shown above it (l. c., pp. 47—52, figs. 16—25). Both of them have 17 bivalent chromosomes, but due to incomplete conjugation in F_1 of the hybrid were single chromosomes occasionally eliminated. *V. velutina* arose comparatively constant in F_3 by loss of one pair of chromosomes (which contained an inhibitor for flower size and the M_1-gene inhibiting *velutina*); it is recessive to *arvensis*. In all other characters than flower size and colour and the chromosome number it resembles *arvensis*. Fig. 134 shows a heterotypic metaphase; one or two pairs of chromosomes have a slight tendency for non-conjugation. *V. velutina* has been cultivated to F_8 of the original crossing and behaves as a completely constant species; it is fairly fertile, a plant of the last generation gave abt. 650 seeds by self-fertilisation.

Cross 11: *V. tricolor alba* × *Kitaibeliana*, $n = 18$.

$[a_1a_1a_2a_2\,..\,llwwSS(f_1f_1\,..) \times A_1A_1A_2A_2A_3A_3LLWWss(F_1F_1F_2F_2).]$

The F_1-hybrid, seven plants, was small flowered as *Kitaibeliana*, pale violet and extremely sterile; the average number of seeds per flower were 0,4. From four plants only 134 seeds were obtained giving 31 F_2-plants, while 33 seeds won by backcrossing to *tricolor alba* gave 21

plants. These plants were just enough to get one yellow flowered F_2-plant, showing that *Kitaibeliana* contains the gene *L* for yellow similar to *arvensis*. The segregation is given below:

		F_2		$F_1 \times$ *tricolor alba*	
anthocyanous	violet	8	31	8	21
	whitish to pale violet	22		12	
	yellow	1		—	
green, *alba*		—		1	

There were no *alba* plants in F_2 but one in the backcross, suggesting that at least three polymeric, basal genes for anthocyanin are present in *Kitaibeliana*. All F_2-plants were small flowering and of *Kitaibeliana*-type, while in the backcross four large flowering plants were found in addition to 17 intermediate. This may suggest that the small flower size of *Kitaibeliana* as compared with *arvensis* is due to the presence of one pair more of principal inhibiting genes, *F*, for flower size. F_2 was largely sterile. There were 26 plants with spot on style in F_2 and five without.

135

Fig. 135. Flower, leaf and stipules of *tricolor* × *rothomagensis*, F_1.

Cross 13. V. tricolor alba-yellow × *rothomagensis* (*violet*).
$[a_1a_1a_2a_2\,..\,(vv)LLwwss \times A_1A_1A_2A_2A_3A_3(VV)llWWSS.]$

The formulæ for this crossing written above are only tentative as regards the *V*-, *L*- and *W*-genes. If consideration had not to be taken to Cross 16, which indicates that *rothomagensis* is homozygous *WW*, and to some later generations of the present cross, the segregations could be explained in a less complicated manner.

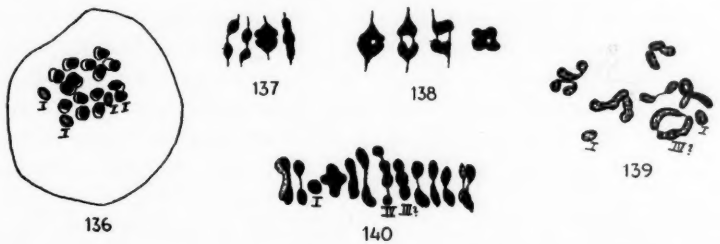
F_1, 34 plants from 47 seeds, was a very long- and thin-stemmed type with stiff and straight, ciliate hairs (fig. 135) reminding much about *rothomagensis* itself. The young flowers were whitish but changed to light or pale violet. It was extremely sterile, and the few seeds obtained germinated very poorly as seen below:

	seeds per capsule	total of seeds	full-grown plants
F_1 selfed	3—4	99	15
$F_1 \times$ *tricolor alba*-yellow	5—7	414	41

The plants obtained were too few for a proper explanation as to the complicated segregation of flower colour, but violet and yellow and a series of pale violet to yellow were obtained as seen below:

		F_2		$F_1 \times$ *tricolor* *alba*-yellow	
anthocyanous	violet	4	15	5	41
	pale violet to whitish	10		19	
	yellow	1		13	
green	*alba*	—		2	
	alba-yellow	—		2	

It is noteworthy that real violet plants were segregated in the backcross $F_1 \times$ *tricolor* yellow; a corresponding cross but with *tricolor* supplying the violet in F_1 would never give violet plants in backcrosses to yellow; similarly, in F_4, a dark violet flowered plant segregated 26 dark violet to eight yellow. These segregations suggest that *rothomagensis* contains the epistatic *V*-gene for violet flower colour, and this gene is not much inhibited by the bleaching *W*-gene. As for the



Figs. 136—140. Chromosomal conditions in later generations of Cross 13. *136—138*: F_2-plant with a very large chromosome (probably aggregation of two); *136*: het. met., 13_{II} (one large) $+ 4_I$; *137*: the large and two ordinary chromosomes in side view; *138*: the large chromosome from four different pollen mother cells, the one most to the right in polar view. *139—140*: an F_3-plant, *139* shows chromosomes from a diaphase with a large, ringshaped tetrasome; *140*: het. met., abt. $11_{II} + 1_{IV} + 1_{III} + 1_I = 30$.

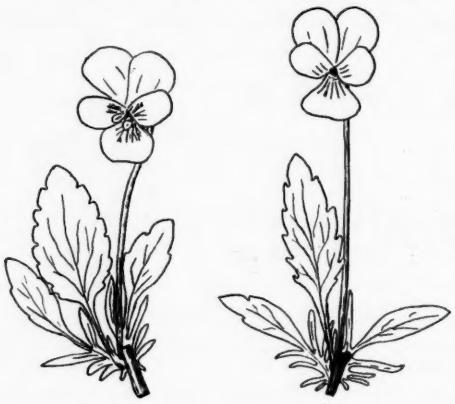
segregation of the characters anthocyanin and dark style spot, see Chapter VI.

A large chromosome probably of double nature was observed in an F_2-plant (figs. 136—138), a constant pale yellow plant giving in F_3 66 pale yellow flowered plants. The big chromosome was twice the size of the others and somewhat bipartite, and in polar view it often showed a curious perforation in the middle (fig. 138, right). It may have arisen through a close union of two and two chromosomes, for a similar chromosome is not seen in any of the parents. It suggests that chromosomes may sometimes aggregate. Figs. 139—140 show diaphase and heterotypic metaphase from a dark violet F_2-plant, which seg-

regated yellow flowered plants in F_4; a real tetrasome was almost invariably present here. Sometimes this tetrasome would resemble a bivalent, but very large, ringshaped chromosome; in other cells, however, the four were attached end to end (fig. 140).

Cross 14: *V. tricolor hortensis* × *lutea.*
[*ll*(*pal pal*), no spot × *LL*(*Pal Pal*), with spot.]

The *Viola lutea* type applied is not intense yellow on the lower petal, but it is not whitish either, as in *V. arvensis.* In F_2 of the crossing were plants segregated, which were much more intense yellow than the *lutea* parent, and this suggests that *lutea* possesses and *tricolor* lacks a bleaching gene of minor order than *W*; it has been named *Pal.*



141 142

Figs. 141—142. Flower, leaf and stipules of F_1. *141*: *tricolor* × *lutea*, trace of doubleness in flower; *142*: *tricolor* × *Battandierii.*

A total of 90 hybrid seeds were obtained and 36 F_1-plants were grown from two crosses; 14 of these belonged to the reciprocal cross *V. tricolor hortensis* × *lutea* and were good and vigorous plants showing characters from both parents; the leaves were larger and more long ovate than in *V. lutea* (see fig. 141), an influence from the very vigorous *tricolor hortensis.* The other 22 F_1-plants represented the weak hybrid *tricolor alba* × *lutea*, of which almost all plants died young (by »black leg«); only four F_2-plants of this last cross reached flowering stage. The F_1-flowers of the cross *tricolor hortensis* × *lutea* were larger than in *lutea* but of similar shape and also streaked as in this species. The colour of the lower petal was pale yellow as in its *lutea* parent, that of the upper ones faint mauve. The fertility was not good, 3—4 seeds per capsule by self pollination; the richly flowering plants could produce as many as abt. 300 seeds, but only a little more than 10 % germinated, giving in all 91 F_2-plants from abt. 800 seeds.

The segregation for flower colour is shown below:

lower petal	observed		calculated	
pale yellow *L Pal*	33	68	68,3	
intense yellow *L pal*	35			total: 91
violet *ll Pal* + *ll pal pal*.....	23		22,7	

The upper petals of the yellow types may have some violet in them.

It was unexpected that segregation did not take place at all regarding spot on style; all 91 F_2-plants had a very distinct spot. This may be due to polymery (multiple genes, *V. lutea* is octoploid!), but spot on style did not show true polymery in any other cross. More probably it is due to duplication of univalents by splitting as mentioned before in chapter IV, perhaps in connection with differential viability.

As regards other characters segregation was evident, also as to the vegetative type, whether similar to *tricolor* or to *lutea*. Two F_3-cultures with 85 and 41 plants, respectively, were constant with spot as F_2. The first one was constant yellow, the last also constant yellow but segregating *velutina*. The general appearance of the F_3 cultures was very uniform and similar to *lutea*.

Cross 15: *V. tricolor hortensis* × *Battandierii*.

This cross was reciprocal and no difference could be observed between the two reciprocals. In fact, F_1 is very uniform, although the chromosome number of the *Battandierii* type oscillates. 31 crossed seeds gave 15 vigorous and very rich flowering plants. F_1 has a large end lobe of stipules and flower shape similar to *Battandierii*, but its size is smaller (fig. 142). KRISTOFFERSON (1923) gave a description of this specific hybrid, of which he cultivated F_1 and F_2. Similar to *Battandierii* are the peduncles of F_1 jointed with the stem. 79 F_2-plants from 313 seeds were all violet flowering as expected.

Cross 16: *V. arvensis* × *rothomagensis*.

[yellowish white *vvLLWW* × violet *VVllWW*, *BBFFss* × *bbffSS*.]

As mentioned under Cross 13 the formula for flower colour of *rothomagensis* is tentative only. The *W*-gene has been attributed to it in order to explain that no yellow plants are segregated in F_2 of the present cross.

The reciprocal cross will not germinate, while 80 seeds of the present combination gave 63 F_1-plants. The chromosome number, it

will be remembered, is the same for both species, viz. n = 17. F_1 was whitish to pale violet flowering with rather small flowers (fig. 143); it had a very faint labellum (almost none) under the stigma, a dark spot on the style and was rather hispid. The fertility was good, as it gave abt. 1500 seeds from five plants, and 542 F_2-plants were raised from them.

F_2 showed fairly clear segregations for several characters (see segregation for size of flowers, for labellum and for spot on style in Chapter VI). As for flower colour, dark violet types (as *rothomagensis*)

Fig. 143. Flowers of Cross 16, *arvensis* × *rothomagensis*. Upper row from left: *arvensis*, F_1 and *rothomagensis*; under them a series of F_2-types. Second row from above: large flowered types, two violet, two whitish and two *velutina* aberrants; third row: small flowered and intermediate types, two violet, two whitish and two violet *velutina* aberrants; bottom row: aberrants, namely violet petaloid, *velutina* petaloid, peloric and petaloid type in frontal and in side view, a type with four sepals petaloid, and finally a small peloric type.

were separable from the other range of variation covering all transitions from yellowish white (similar to *arvensis*) to pale violet; the enumeration is given below:

	observed		calculated	ratio
(WW)L.vv, yellowish white	435	516	419,3	13
(WW)L.V. + *(WW)llvv*, pale violet				
(WW)llV., violet	81		96,7	3

Fig. 143 gives an impression of the variation in F_2 from yellowish white to violet, and from small to large flowers. It shows also the flowers of some of the aberrant types, which appeared in great number (see Chapter VI): *velutina*, petaloid, and also peloric types with spur on lateral petals. Sterile or semisterile plants were frequent in F_2 (see Chapter VI, aberrant types). They were just of the type described from Cross 9, *tricolor* × *arvensis* (J. CLAUSEN 1926, 1927 a), the leaves were entire but often necrotic and variegated due to local absence of green palissade tissue (see fig. 144 and compare it with figures in the

Fig. 144. Sterile type of Cross 16 (with entire leaves); white-variegated by degeneration of palissade tissue.

paper cited above and with fig. 146 of Cross 19 in the present paper). *Viola rothomagensis*, when crossed with *arvensis*, gives accordingly the same aberrant types as the cross *tricolor* × *arvensis*. This should indicate some genic or constitutional similarity between *rothomagensis* and *tricolor*, but the fertility test shows *rothomagensis* to be much more related with *arvensis* than with *tricolor*.

There was found a similar linkage between the inhibitors for flower size and for labellum as previously noted in the cross *tricolor* × *arvensis*, but this is not surprising as the inhibitors in both cases came

from *arvensis*; the numbers of the four combinations were the following:

flower size	labellum	observed	
small (*FF*) + intermediate (*Ff*)	no labellum (*B.*)	343	516
	with labellum (*bb*)	33	
large (*ff*)	no labellum (*B.*)	24[1]	
	with labellum (*bb*)	116	

Plants with large flowers and without labellum were generally yellowish white flowering, and the sepals of such plants were also large, almost of the same length as the petals.

Cross 17: V. Kitaibeliana n = 7 × arvensis, Line 52.

Except as regards chromosome number these two species are very alike. The most striking difference is in shape and size of flower as seen from fig. 5. F_1 in this respect resembled its *arvensis* parent, while the characters of the leaves were more like *Kitaibeliana.*

About 1100 seeds from two F_1-plants yielded no more than 197 F_2-plants, and these had all without exception the open, flattened shape of corolla similar to their *arvensis* parent. For numerous small characters were segregations noted: mode of branching, length of nodes (1,5—4 cm.), shape of stipules, dentation of leaves (crenate, serrate, somewhat incised), shape of leaves (ovate, spathulate, lanceolate; broad, narrow, leaves of most plants were narrow as in *Kitaibeliana*), thickness of stem, size of flower (varied somewhat, but mainly as in *arvensis*), corolla-limb (broad or narrow, more or less flattened, never of the closed cup-shaped type as in *Kitaibeliana*) and finally the colour of the flower (yellowish white to faint mauve). There were also great variation as to fertility, ranging from absolute sterility to abt. 200 seeds on rather small plants.

The relative constancy of some *arvensis* characters may depend upon the behaviour of the chromosomes in F_1, where many univalents (mainly *arvensis* chromosomes) split. The average number of chromosomes in F_2 appears to be above that of F_1 ($2n = 24$), tending to go in direction towards the *arvensis* parent ($2n = 34$) or even above it. In nine F_2-plants the chromosome numbers were the following:

$2n =$ abt. 21, 24, 26, 27 or 28 (two plants), 28, 29, 31, abt. 36.

A semi-sterile plant with entire leaves (cf. Crosses 16 and 19) was segregated. Neither of the parent species had spot on style, and spot was not syntethizised through the crossing.

[1] 21 of these were yellowish white!

Cross 19: V. Kitaibeliana n = 7 × Kitaibeliana n = 18.

This cross was made in both directions, and 13 F_1-plants were secured from 93 crossed seeds. Fig. 145 shows the F_1 between its two

Fig. 145. Cross 19; from left: *Kitaibeliana* n = 7, F_1, *Kitaibeliana* n = 18.

parents. It was extremely sterile, and as the seeds were very few and *Kitaibeliana* otherwise is a good selfpollinator, it was thought safe to use also seeds from some few capsules set before the plants were removed to the insect-proof green house. A total of abt. 500 seeds were collected from five F_1-plants. This gave only 33 F_2-plants and of these abt. 15 were apparently vicinists, triple hybrids from open pollination. 18 plants apparently came from selfpollination and had small flowers with closed, not flattened limbs and yellowish white or faintly mauve colour of flowers.

Fig. 146. Sterile type with entire leaves, segregated in F_2 of Cross 19.

Almost all hybrid, vicinistic seeds may have germinated, while of the real F_2-seeds only abt. 3,6 % were able to do so. The experiment thus shows that when a highly sterile hybrid under natural conditions is exposed to open pollina-

tion, then formation of triple hybrids or backcrosses will be favoured more than selfpollination. This is because the pollen from the hybrid is not so good as from a foreign pure species, and seeds containing at least one complete, balanced set of chromosomes germinate much better than do seeds containing only recombined and exchanged chromosomes from two specific different sets. As well the supposed vicinistic plants as the true F_2-plants were almost completely sterile. One plant of the non-flowering sterile type with entire leaves appeared in F_2 (fig. 146).

Cross 22: *V. lutea* × *Battandierii*.
[pale yellow, *LL*(*Pal Pal*) × *ll*(*pal pal*), violet.]

These species are doubtless somewhat related, and crossing is easily effected. 40 seeds giving 22 F_1-plants were obtained from it. F_1 reminds vegetatively about *Battandierii* (also in its flower shape) but the flower colour is more pale violet and the lower petal is faintly yellowish.

About 250 seeds were obtained by selfpollination, and these gave 131 F_2-plants. Many gradations as to colour of flowers were noted, but a number of different shades can also be produced by the interaction of the two genes suggested above. Some of the yellow types were yellow on the lower petal only, faint violet on the upper ones; others were pale yellow on all petals, and one was noted, which was intense yellow on all five petals as the cultivated *V. lutea grandiflora*. The enumerations as to flower type of F_2 are given below:

flower colour	observed		ratio	calculated
violet . . . . . *ll*	43		1	
± pale violet . . *Ll*	52	131	2	98,3
yellow, ± intense *LL*	36		1	32,7

A wide range of variation was noted in F_2 as to growth type (cespitose or with very long side branches), size of flowers, length and shape of spur (straight or curved) and length of peduncle (one plant had peduncles as long as 15 cm.). Ten plants had abnormal style, either a bilateral or even a three-sided, actinomorphic one.

Cross 24: *V.* (*tricolor alba-yellow* × *Orphanidis*) × (*cornuta* × *elegantula*).

Although *V. tricolor* cannot be crossed directly with *cornuta*, this present quadruple hybridisation was easily effected. HERIBERT NILSSON (1930) had similar experiences in his multispecific hybrids of *Salix*.

Fig. 147 shows the pedigree of Cross 24. F_1, V. 1205, consisted of 39 plants and due to its being F_1 of a quadruple hybrid it showed a considerable variation (see fig. 148). This applies to flower shape (reminding about *cornuta, elegantula-Orphanidis* or even *Battandierii*), direction of lateral petals (horisontal or turned upwards, never downwards), colour of flowers (dark violet to very light or pale violet and reddish violet), length and shape of spur (straight or curved), extension of the yellow eye present in all plants, shape of style, shape of stipules

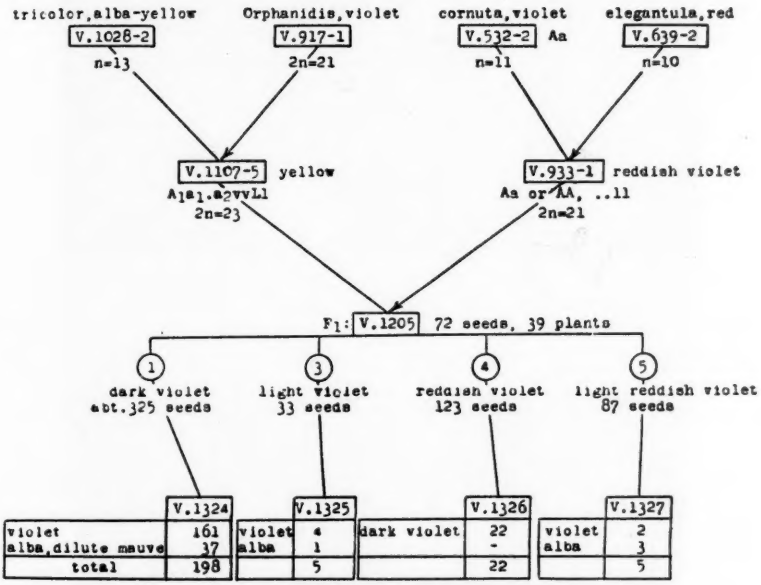


Fig. 147. Diagram of Cross 24 (quadruple).

(triangular, lyrate-pinnatifid, palmate; degree of division also varying) and the character of the ramification, whether cespitose or more open. The fertility was also extremely variable.

Different types of segregation were also obtained in the individual F_2-sowings (V. 1324—1327, fig. 147) according to from which of the four grandparental species the chromosomes were derived, which constituted the corresponding F_1-plant in V. 1205.

Fig. 149 shows flowers of V. 1324. This F_2-sowing contained 198 plants; the general appearance of them was similar to *cornuta*; this applies decidedly to leaves and stipules. The shape of flowers

corresponded to Cross 5 (*Orphanidis* × *cornuta*), but some plants suggested a *tricolor* parent. The erect growth was derived from *tricolor*.

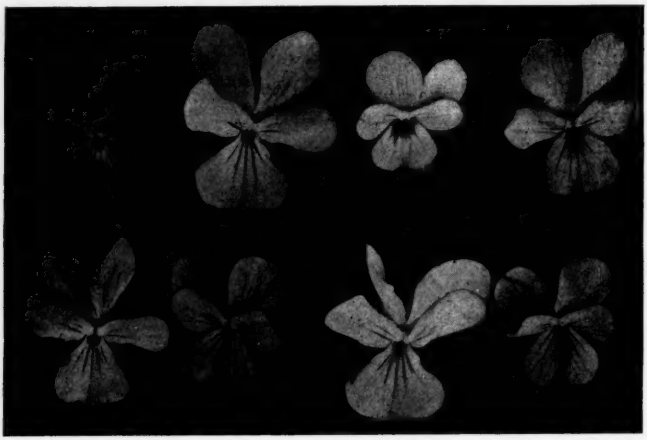


Fig. 148. Types of flowers in Cross 24, F_1, V. 1205 (portrait film).

The colour of the violet flowers was somewhat darker than in the *Orphanidis* × *cornuta* cross and more reddish (influence from *elegantula*?). The yellow eye could originate from any of three parents. There were large variation as to extent of yellow in the eye, and one

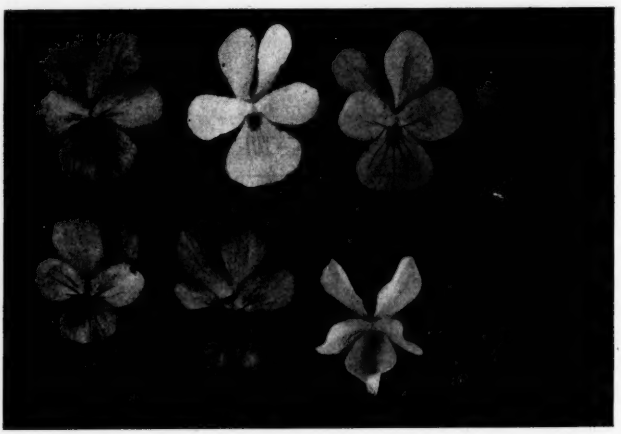


Fig. 149. Types of flowers in V. 1234, F_2 of quadruple hybrid. Note difference as to yellow eye, direction of lateral petals and shape of petals (portrait film).

plant had a totally white eye (fig. 149, a *cornuta*-character). The segregated green-stemmed, non-anthocyanous plants were not *alba*-flowering but with a tinge of dilute mauve; this colour is not noted from any of the parents before; the non-anthocyanous condition could be inherited from either *tricolor* or *cornuta*. The direction of the lateral petals varied from horisontal or slightly turned upwards to downwards turned as in *cornuta*. The flower size was very variable, the spur of all plants was thin and straight, but in no case it attained a length as in *cornuta*. Some were hirsute as *Orphanidis*, others almost glabrous. Apparently, therefore, this sowing showed characters from all four parent species.

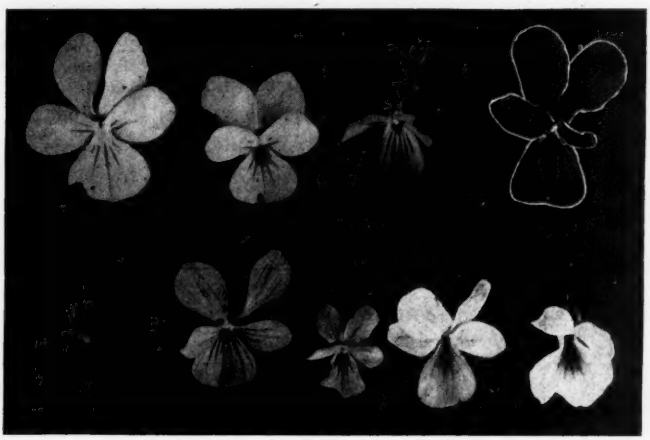


Fig. 150. Cross 24, F_2-types of flowers. Upper row from left: two flowers of V. 1325; the two to the right and the three left ones in lower row are of V. 1326; right in lower row: two *alba* flowers of V. 1327, the right one is peloric (portrait film).

V. 1325 resembled *tricolor* as to its flower shape (fig. 150); two of a total of five plants had flowers with white eye (no yellow); the stipules were as in *cornuta* or as in *elegantula*.

V. 1326 contained only dark reddish violet flowering plants, two of them were much darker than any of the parents (fig. 150); four plants were cespitose, non-flowering dwarfs (in addition to 22 flowering plants); the flower shape reminded about *elegantula* and *Orphanidis* and the stipules varied from a shape similar to those of *elegantula* to a triangular shape as in *cornuta*. Most characters in this sowing point towards *elegantula*.

V. 1327 contained three pelories among a total of five flowering

plants (fig. 150); these pelories were all *alba*-flowering. The stipules were of *elegantula* type with some influence from *cornuta*.

It appears as if the presence of chromosomes from four different sets do not cause any serious inflictions.

VI. SEGREGATION OF SOME CHARACTERS.

While the flower colour shows such complicated inheritance that it was thought better to deal with it under the individual crossings, some of the other characters may more practically be dealt with for more hybrids simultaneously.

THE BASAL GENES FOR ANTHOCYANIN COLOURS (A-SERIES).

Table 13 shows that *elegantula*, *Orphanidis* and *cornuta* all carry one basal gene for anthocyanin. Cross 7 gives too few *alba*, but there

TABLE 13. *Segregation of A-genes.*

being analysis of		*elegantula* n = 10				*Orphanidis* n = 11, 10				*cornuta* n = 11	
Cross no.		Cross 1: *corn. alba* × *elegant.*		Cross 6: *tric. alba-y.* × *elegant.*		Cross 5: *Orphan.* × *corn. alba*		Cross 7: *tric. alba-y.* × *Orphanidis*		*cornuta*, violet × *alba*	
formulæ		$\frac{a_1}{A_1}$		$\frac{a_1a_2}{A_1.}$		$\frac{A_1}{a_1}$		$\frac{a_1a_2}{A_1.}$		$\frac{A_1}{a_1}$	
		obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
F_2	antho-cyanous	42	42,7	6	4,5	113	106,5	163	158,2	106	105,0
	alba	15	14,3	—	1,5	29	35,5	15	19,8	34	35,0
	total	57	57,0	6	6,0	142	142,0	178	178,0	140	140,0
	ratio		3 : 1		3 : 1?		3 : 1		8*A* : 1*a*?		3 : 1
backcrosses	antho-cyanous	17	16,0			—	—	95	94,7		
	alba	15	16,0			—	—	47	47,3		
	total	32	32,0			—	—	142	142,0		
	ratio		1 : 1						2*A* : 1*a*?		
F_3	antho-cyanous					160	144,0				
	alba					32	48,0				
	total					192	192,0				
	ratio						3 : 1?				

are also too many for a 15 : 1-ratio. The same *Orphanidis*-parent, viz. V. 917—1, was applied for both Cross 5 and Cross 7. Cross 5 gives a 3 : 1 ratio, and as it is a less wide cross than 7, it is more conclusive than this latter; Cross 5 refers to five F_1-plants. In Cross 7 would a gametic ratio of $2A : 1a$ (by gametic sublethality) explain the segregation as well in F_2 as in the backcrosses. But other characters show almost normal segregation (for instance violet : yellow), and zygotic sublethality of the *alba* type may therefore be more probable. The ratio is bettered in F_3.

Wild growing types of *tricolor* and *alpestris* have two basal genes for anthocyanin, while the old garden type of *tricolor* (as well *velutina* 3, as *tricolor nigra*) has three genes and segregates 63 : 1 (as *arvensis*). It will be remembered that *alpestris* and *arvensis* both contain the *D*-gene for dilute mauve.

TABLE 13 (continued). *Segregation of A-genes.*

being analysis of		*tricolor* n = 13				*alpestris* n = 13		*arvensis* n = 17		*Kitaibeliana* n = 18	*rothomagensis* n = 17
		wild types		*hortensis*							
Cross no.		*tricolor*, coloured × *alba*		*tricolor*, *hortensis* × *alba*		Cross 8: *tric. alba* × *alpestris*		Cross 9: *arvensis* × *tric. alba*		Cross 11:	Cross 13:
formulæ		$\frac{A_1A_2}{a_1a_2}$		$\frac{A_1A_2A_3}{a_1a_2a_3}$		$\frac{a_1a_2}{A_1A_2}$		$\frac{A_1A_2A_3}{a_1a_2 .}$		$\frac{A_1A_2A_3(?)}{a_1a_2 .}$	$\frac{a_1a_2 .}{A_1A_2A_3}$
		obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	obs.
F_2	anthocyanous	5200	5132,8	2147	2147,9	801	790,3	1054	1050,3	31	15
	alba	275	342,2	35	34,1	42	52,7	13	16,7	—	—
	total	5475	5475,0	2182	2182,0	843	843,0	1067	1067,0	31	15
	ratio		15 : 1		63 : 1		15 : 1		63 : 1	63 : 1?	63 : 1?
					anthocyanous			376	371,9	20	37
			backcrosses		*alba*			49	53,1	1	4
					total			425	425,0	21	41
					ratio				7 : 1	7 : 1?	7 : 1
									anthocyanous		30
								F_3	*alba*		1
									total		31
									ratio		63 : 1?

Abbreviations: *alba*-y. = *alba*-yellow; *corn.* = *cornuta*; *Orphan.* = *Orphanidis*; *tric.* = *tricolor*; calc. = calculated; obs. = observed.

The numbers are too small in Crosses 11 and 13 for an exact analysis of *Kitaibeliana* n = 18 and *rothomagensis*, respectively; but the few segregated *alba* individuals seem to indicate at least three *A*-genes in both species, just as in the third hexaploid species, viz. *arvensis*.

Provided the distribution of univalent chromosomes in F_1 is at random and free (that is to say: surplus chromosomes from the species with the largest chromosome number do not unite autosyndetic, nor polysomic, and are not eliminated to any considerable degree) the segregation of dominant genes present in the univalent chromosomes should not deviate considerably from the ordinary Mendelian ratio, because only the dominant genes count and recessive genes count equal to absence of the corresponding chromosome. When a species shows a recessive character, say green stem, it can have no dominant genes (*A*) at all for this character present in any of its chromosomes, all its chromosomes are, so to say, recessive as to *A*, being *a*.

When the species with the highest number of chromosomes is that with the dominant *A*-genes, elimination of univalent chromosomes with any of these genes may bring the segregation down to a seemingly lower degree of polymery; and if univalent chromosomes containing any *A*-genes split, or if autosyndesis takes place, the hybrid may simulate a segregation according to a higher degree of polymery or eventually behave as constant.

It appears as if there is a close coincidence between the degree of polymery and the degree of polyploidy within the *Melanium* section, when it is remembered that *elegantula*, *cornuta* and *Orphanidis* are regarded as diploid members of a 10-series, while *tricolor* and *alpestris* are tetraploid, and *arvensis*, *Kitaibeliana* 18 and *rothomagensis* are hexaploid members of a 6-series.

Not many analyses have been made of the distribution of genes in hybrids between species differing as regards chromosome number and as regards polymery. One of the most interesting is the cross *Euchlæna perennis*, n = 20 × *Zea Mays*, n = 10 (R. A. EMERSON 1929). Crosses were made in such a way that comparison could be made between segregations, where Maize (the diploid parent) carried the dominant genes, *Euchlæna* (tetraploid) the recessive ones, and, on the other hand, such segregations, where the tetraploid *Euchlæna* carried the dominant genes. *Euchlæna*- and Maize-chromosomes appear to be close homologous; the test showed, in fact, that Maize carried one set of genes, *Euchlæna* at least two sets for each of the four dominant

characters investigated. When Maize was used as the dominant parent, the backcross of F_1 to recessive Maize showed a true monohybrid segregation, giving 341 of the dominant type and 401 of the recessive one (a 1 : 1 ratio). On the other hand, when *Euchlæna* was the dominant parent and the F_1-hybrid similarly was backcrossed to recessive Maize, only very few recessives were segregated, namely 29 recessives as compared with 375 dominants; this is even far below the expected dihybrid ratio 3 : 1 and also far below a trisomic ratio 5 : 1, being more like a 15 : 1 segregation. The ratio probably indicates a certain percentage of autosyndesis between dominant *Euchlæna* chromosomes. Such autosyndesis has no visible effect, when the *Euchlæna* chromosomes carry the recessive genes.

The segregations in *Viola* indicate in almost all cases a free assortment of the chromosomes carrying the polymeric (multiple) genes, but in those cases, where the dominant species has the largest number of chromosomes, one of the polymeric genes may be without a mate.

FLOWER SIZE AND PRESENCE OF LABELLUM.

These two characters are by taxonomists regarded as taxonomic significant ones and justly so. It is therefore of some interest that also specific characters segregate after laws similar to varietal characters. This is dealt with previously for one hybrid (J. CLAUSEN 1926). Here is only given a survey on these segregations (see table 14).

Several genes are no doubt responsible for flower size and for labellum, but the segregations seem to indicate at least one gene with a superior effect of inhibiting the flower size. The segregation is clearest, when the genes of minor importance modifying the effect of the inhibiting gene are absent. Cross 11 suggests at least two inhibiting genes for flower size and for labellum in *Kitaibeliana* n = 18, which is very small flowered. There is linkage between the inhibitors for flower size and for labellum (J. CLAUSEN 1926 and Cross 16).

YELLOW EYE.

It has been mentioned that yellow eye is a character of superior taxonomical value. The segregation of this character is shown in table 15.

It is obvious from the types segregated, of which some are shown in figs. 148—150, that genes also for the extension of the eye are present, and the entire genetical basis for this character may be a very complicated one.

TABLE 14. *Segregation of flower size and labellum in different crosses.*

Cross			Cross 9: *tricolor* × *arvensis* (XI, 1926)		Cross 11: *tricolor* × *Kitaibeliana* n = 18		Cross 16: *arvensis* × *rothomagensis*		
flower size			observed	calculated	observed		observed		calculated
	small + intermediate	F_2 + F_4	243	236,3	31		376		387,0
	large		72	78,7	—	F_2	140	F_2	129,0
	total		315	315,0	31		516		516,0
	ratio			3 : 1	15 : 1?				3 : 1
backcross, F_1 × large flowered	flower size		(XII, XIV, XV 1926)						
	intermediate		538	479,5	17				
	large		421	479,5	4				
	total		959	959,0	21				
	ratio			1 : 1	3 : 1?				
labellum									
	without	F_2 + F_4	215	219,0	16	backcross to *tricolor*	367	F_2	387,0
	with		77	73,0	5		149		129,0
	total		292	292,0	21		516		516,0
	ratio			3 : 1	3 : 1?				3 : 1

TABLE 15. *Segregation of yellow eye.* (F_2).

Crosses	Cross 1: *cornuta* × *elegantula*		Cross 5: *Orphanidis* × *cornuta*	grand total
eye	observed	calculated	observed	
yellow	73	66,0	127	200
white	15	22,0	15	30
total	88	88,0	142	230

Back cross (*cornuta* × *elegantula*) × *elegantula* gave 27 plants, all with yellow eye.

STYLE SPOT.

See notes on the inheritance of this character on p. 242. Table 16 shows its segregations.

TABLE 16. *Segregation of the character style spot in the individual crosses (all showing a 3 : 1 ratio).*

Crosses formulæ:	Cross 8: *alpestris Ss* × *tricolor SS*		Cross 11: *Kitaibeliana* 18 × *tricolor* $\frac{s}{S}$		Cross 13: *tricolor* × *rothomagensis* $\frac{s}{S}$		Cross 14: *tricolor ss* × *lutea SS*			Cross 16: *arvensis* × *rothomagensis* $\frac{s}{S}$	
generation	F_2		F_2		$F_2 + F_3 + F_4$					F_2	
	observed	calculated	observed	calculated	observed	calculated	F_2	F_3	total	observed	calculated
with spot	143	156,0	26	23,3	82	85,5	91	127	218	385	387,0
no spot ...	65	52,0	5	7,7	32	28,5	—	—	—	131	129,0
total	208	208,0	31	31,0	114	114,0	91	127	218	516	516,0

ABERRANT TYPES.

It is striking that there are certain aberrant types, which segregate again and again from different specific crosses. Table 17 gives a survey on their segregation.

TABLE 17. *Aberrant types segregated in F_2 of different specific crosses.*

Crosses	Cross 6: *tricolor* × *elegantula* backcross		Cross 8: *alpestris* × *tricolor* F_2		Cross 9: *tricolor* × *arvens.* 6 crosses	Cross 16: *arvens.* × *rothomag.*	Cross 17: *Kitaib.* 7 × *arvens.*	Cross 19: *Kitaib.* 7 × *Kitaib.* 18	Cross 22: *lutea* × *Battand.*
types	F_1 × *tric.*	F_1 × *eleg.*	*A*	*B* + *C*	F_2	F_2	F_2	F_2	F_2
normal	34	67	461	1971	4423	436	196	19	119
sterile dwarfs	24	23	—	—	75	26	1	1	—
petaloids	—	—	—	53	70	22	—	—	2
pelorics	—	—	—	—	88	23	—	—	10
velutina	—	—	2	—	52	35	—	—	—
total	58	90	463	2024	4708	542	197	20	131

Cross 7. In F_3 of *tricolor* × *Orphanidis* appeared two *velutina* aberrants.

The aberrants from different crosses were often so similar in type, that they would be difficult to tell apart, if being mixed with each others. The sterile dwarfs in Cross 6, only, were different from other dwarfs, inasmuch as they were not of the necrotic-variegated type; some of them were very minute, cespitose dwarfs without any signs of flowering at all.

No doubt the chromosomes of the *Melanium* section are largely very homologous as to genetic structure, and certain losses, therefore, create similar aberrants. Some of the aberrants are probably due to losses of entire chromosomes, but deficiencies and real mutations may also have taken place, although they never occur in the pure species cultivated.

VII. CONCLUSIONS AND SUMMARY.

In the following the general biological facts substantiated and dealt with in some detail in the foregoing will be extracted and summarized.

HOMOLOGY OF CHROMOSOMES.

It is characteristic for the *Melanium* violets that all specific hybrids show some amount of chromosome conjugation. There is a tendency that so many bivalents are formed as corresponds to the haploid chromosome number of the parent with the smallest number of chromosomes; that is to say, such number of bivalents is maximum, but in most cells one or two pairs less are formed. Some specific hybrids vary so largely as to chromosomal conjugation that cells are observed without any conjugation of chromosomes at all, but they show a great range of variation in the number of univalents from cell to cell (Cross 7: *tricolor* × *Orphanidis* and Cross 11: *tricolor* × *Kitaibeliana* n = 18). Cross 18 (*nana* × *arvensis*, n = 24 × 17) shows comparatively the smallest amount of conjugation of any of the hybrids.

In three crosses were more bivalents formed than the species with the smallest chromosome number allowed; these were Cross 3 (*lutea* × *elegantula*, 24 × 10), Cross 12 (*tricolor* × *nana*, 13 × 24) and Cross 15 (*tricolor* × *Battandierii*, 13 × 26—30). A species with a high number of chromosomes enters in all these crosses, and excess bivalents are no doubt formed by autosyndetic union of chromosomes from the species with the high chromosome number. But these species do not always give autosyndesis. Cross 14 (*tricolor* × *lutea*) shows the number of univalents stipulated by the difference between the numbers of the two species; Cross 20 (*nana* n = 24 × *Kitaibeliana* n = 7) does not

show any autosyndetic union of the 17 extra *nana* chromosomes, although they unite in Cross 12; and in Cross 18 (*nana* × *arvensis*, n = 24 × 17) the *nana* chromosomes are obviously neither capable for conjugation with themselves nor with those of *arvensis*.

The polysomic arrangement of chromosomes is mainly found in the hybrids, in which *elegantula* enters as one parent (Crosses 1, 2 and 6 and also in Cross 3). The hybrids of *rothomagensis* show a tendency, though not so striking, for formation of trivalents and, rarely, quadrivalents (Crosses 13 and 16), and the hybrids with *lutea* contain often irregular chains of chromosomes (Crosses 3, 14 and 21).

If these phenomena should be interpreted in the manner current in recent cytological philosophy, one would say that the chromosome complements of the *Melanium* species are built up regularly from the base in a manner that one set of chromosomes is present in all species, and the set added in tetraploids is present also in all hexaploids and octoploids; the extra set added in the hexaploids should furthermore be present twice in the octoploids. The following scheme gives a picture of the chromosomal complements of the species belonging to the subsection *Tricolores*, and each letter indicates a set of approximately six chromosomes:

diploid: $\frac{A}{A}$ (*Kitaibeliana* n = 7),

tetraploid: $\frac{AB}{AB}$ (*tricolor, alpestris,* n = 13),

hexaploid: $\frac{ABC}{ABC}$ (*arvensis, rothomagensis,* n = 17; *Kitaibeliana* n = 18),

octoploid: $\frac{ABCC}{ABCC}$ (*nana, lutea,* n = 24).

The repeated set in the octoploid species should account for the autosyndesis observed in some of their hybrids. The cytological construction of the *Melanium* subgenus is different from that of *Nicotiana* (R. E. CLAUSEN 1928, KOSTOFF 1930) and from *Triticum* (AASE 1930, WATKINS 1930 and BLEIER 1930); these two genera do not have one basal set of chromosomes present in all of their species. Although some variation occurs in the amount of conjugation in meiosis of hybrids in these two last named genera, the operation of the chromosomes in the hybrids largely suggests definite sets of chromosomes present, in some cases homologous, in other non-homologous or both together. A very well established case of repetition or duplication of at least one set of chromosomes in a polyploid species is the hexaploid *Solanum*

nigrum, the haploid type of which shows abt. 12 bivalents and 12 univalents with a tendency even to the formation of one or two trisomes (JØRGENSEN 1927).

The *Melanium* species with 10 and 11 chromosomes apparently contain at least the same basal set of chromosomes as the *Tricolores* have, and perhaps also a part of the second set (B). But the chromosome set of *elegantula* should furthermore represent a partial reorganisation by segmental interchange between non-homologous chromosomes, not any systematic and regular interchange but a more irregular one. Some of the chromosomes of *rothomagensis* should have undergone a similar reorganisation. This will account for the polysomic association of chromosomes met with in the hybrids of these species with the other ones.

The polymeric (multiple) genes proven to be present in *Viola* species in accordance with the degree of polyploidy of the species (in first line the *A*-genes for anthocyanin) suggest a repetition of a single set of chromosomes in the polyploid species. But many other genes are not repeated in each set, and there is no doubt that many differences exist, also as regards genes, between the individual sets of chromosomes present in the polyploid species. Probably only genes, which are very widespread and perhaps homozygotic present in almost the entire section, and vital significant genes are repeated in each set of chromosomes. Many processes may have furthered the differentiation of chromosomal sets from possibly one original set. Addition, subtraction, duplication and exchange of entire chromosomes or parts of them may account for many deviations from an identical behaviour by chromosomes of species with the same chromosome number.

But although there seems to be some reality behind the theory about homology of chromosomes as indicated by their ability or non-ability for mutual conjugation, such facts are known, which cause great difficulties for this winning and plain theory.

The chromosomes of *arvensis* show their homology with those of *tricolor* by conjugation with *tricolor*'s chromosomes, at least the 12—13 first ones do so. When *tricolor* next is crossed with *V. nana* $n = 24$, 16—18 bivalents are formed, proving the homology of *tricolor*'s chromosomes with a similar number of *nana* chromosomes; but in addition a certain number of *nana* chromosomes must conjugate mutually, which after the theory of homology should prove that *nana* had two homologous sets of chromosomes at least. The same *nana* was crossed with *arvensis* and almost no bivalents at all were formed in this hybrid.

By homology at least 12—13 *arvensis* chromosomes sould be expected to be capable for conjugation with a similar number of *nana* chromosomes, but only 2—6 bivalents were observed, and these may just as well be *nana* chromosomes conjugating mutually, as they did in the *tricolor* × *nana* hybrid. How are we to look upon such discrepancies?

Conjugation of chromosomes no doubt indicates a kind of homology between them (perhaps some identical arrangement of certain genes and their alternatives), but non-conjugation does not necessarily always mean non-homology. Other factors may prevent the conjugation. Non-conjugation in pollen mother cells of pollensterile *V. Orphanidis* and complete conjugation in embryosac mother cells of the same plant (J. CLAUSEN 1930 b) indicated that environment conditions of the cells may prevent conjugation, even if the chromosomes themselves are homologous. The poor conditions in the male archespor was probably due to a certain constellation of genes or chromosomes. The similar asynaptic condition without conjugation of chromosomes in the pollen mother cells of a *Zea Mays* may in its realisation have been produced even by the action of a single recessive pair of genes (BEADLE 1930). The chromosomes conjugate, *if* the individual chromosomes are homologous, and *if* conjugation is not prevented by some unbalance in the total complement of chromosomes of the individual in question. Such unbalance is frequent in specific hybrids.

The present investigation shows how prudent we have to be in drawing conclusions about the origin of chromosome numbers. DARLINGTON and MOFFETT (1930) conclude that the 17-chromosome *Pyrus* belong to a 7-series and have two sets plus one partial set of seven chromosomes. They base this assumption upon the presence of hexasomes and other polysomic associations observed in the types investigated. Their figures afford no conclusive evidence about hexasomes and tetrasomes in the pure species as *Pyrus floribunda* and *P. Ringo*, but they do so for the cultivated types, which are very complicated hybrids. Specific or even varietal hybrids, whose parents had normal conjugation of chromosomes, can produce very complicated polysomic associations of their chromosomes. This was proven to be the case with OSTENFELD's *Polemonium* hybrids (J. CLAUSEN 1931 a), and more hybrids described in the present paper show the same. The polysomic association of chromosomes in the cultivated types of *Pyrus* may therefore just as well have arisen secondary through more or less remote crossings between types belonging to the 17-series.

TAXONOMIC RELATIONSHIP OF SPECIES; SPECIES CONCEPT.

The diagram, fig. 1, of the crossing possibilities of the species subject of this paper shows that *Viola cornuta-orthoceras* and *Viola calcarata* occupy isolated positions in the section; *cornuta* was capable of crossing with *Orphanidis* and *elegantula* only, and *calcarata* likewise with *Battandierii*. Assumed hybrids of *calcarata* with *alpestris* are noted from nature, but these are sterile.

V. tricolor and probably also *arvensis* occupy a central position in the *Melanium* section; at least the first named species forms hybrids with almost all groups of species; it was extensively used for crossing, that is true, but one reason for this extensive use was its ability for crossing. The distribution of the two species and their ability for dissemination over large parts of the world suggest that they are not too specialised, as the alpine species of this section are, and this is perhaps the reason why their genic complement harmonizes with almost all species of their section; they act, so to say, as common denominators of the entire section.

V. Kitaibeliana seems to be more specialised, but the types forming this species are largely incompatible with each others, although the morphological uniformity no doubt also suggests a certain uniformity as to genes determining the morphological type. But their genic complement must contain other elements causing the incompatibility. It is paradoxal, indeed, that one member of this collective species, viz. *nana*, is much more compatible with a morphologically very different species as *Viola tricolor* than the types belonging to the collective species *V. Kitaibeliana* are mutually.

Viola lutea and *Battandierii* are compatible as shown by their hybrid, and the author suggests that *rothomagensis* belongs in their relationship; the crossing *rothomagensis* × *lutea* did not succeed, but the reciprocal one was unfortunately not tried; keeping in mind the difficulty encountered by the crossing *rothomagensis* × *arvensis* but not by the reciprocal one, there is a possibility that the cross *lutea* × *rothomagensis* would succeed. Spontaneous crossings show *rothomagensis* to be very compatible with *Battandierii*.

The position of *Battandierii* has been somewhat unclear. BECKER (1925) draws it to *Calcaratæ*, but its possibilities for crossings with *tricolor* and *arvensis* (KRISTOFFERSON 1923), with *rothomagensis* and with *lutea* place it among the *Tricolores*, because *calcarata* itself is not compatible with these species. The correction as to its chromosome

number in the present paper also stresses its position in the neighbourhood of *lutea* rather than near *calcarata*. But of the *Tricolores* it is the species, which shows the closests relationship to *calcarata*.

It becomes more and more difficult to maintain a criterium for specific difference and specific relationship. The old claim, morphological difference combined with more or less intersterility between specific different types, is not answered to by nature in all cases. Groups of types exist, which on practical grounds by taxonomists must be kept into one species (e. g. *Kitaibeliana—nana*), but which are incompatible by crossing, giving almost sterile hybrids, while one of the types in this collective species (viz. *nana*) is fully fertile with a morphologically very different species, having a widely different chromosome number. FEDERLEY (1928) observed in the generic hybrid *Metopsilus porcellus* × *Chærocampa elpenor* complete conjugation of the chromosomes combined with full fertility and Mendelian segregation in F_2, while the specific hybrid *Pygæra curtula* × *P. pigra* was sterile and did not show any conjugation of chromosomes. This shows that a classification based upon morphological difference alone may often need a correction based upon a cyto-genetic study.

The chromosome number itself often is a support in the botanical taxonomy and classification, but in some cases it fails entirely. The morphological appearance of the species does not depend upon the chromosome number itself. The highly different types belonging to *tricolor—alpestris* (fig. 5, the two right flowers in the upper row and the entire middle row) all have 13 chromosomes, while the uniformly appearing types of *arvensis—Kitaibeliana—nana* (fig. 5, lower row) have widely different numbers of chromosomes, viz. 17, 7, 8, 18 and 24. It is the content of genes, not the manner in which these genes are aggregated, which determines the morphological type. The homology of chromosomes may in some cases be a help in classification, but as previously shown this is not either an unfailing one, when secondary interchange between primarily non-homologous chromosomes must be supposed to have taken place.

It will be impossible to give any definition on the conception of species covering all cases, as attempted by several authors (DU RIETZ 1930, TURESSON 1929, partially also HERIBERT NILSSON 1930). Nature is infinitely much richer than our termini and classifications allow, and the points of view advocated by BABCOCK (1930) without any attempt at a definition of the species seem to the present author to be most in accordance with the conditions shown by nature itself. It might be

convenient and also correct to maintain a merely theoretical conception of species together with one for the practical taxonomy based upon easily observable and safe morphological characters. According to the theoretical conception of species the different chromosomal types of *Kitaibeliana* should be treated as specific different, while the practical taxonomy should treat them as one species. The chromosomal types need no specific names of their own, but may in the present case be named *V. Kitaibeliana* with the chromosome number added just as practised in the present paper. But the *delimitation* and *classification* of species acknowledged in practical taxonomy, as a matter of course have to be established on investigations starting from as many points as possible in order to arrive at the most natural classification and delimitation; such kinds of investigations have to cover variation in field and its relation to ecological conditions and geographical distribution, chromosomal morphology and complement, crossing possibilities and fertility of hybrids, degree and nature of chromosomal conjugation in hybrids and genic accordance.

The *Melanium* violets afford a very good illustration to the difficulties encountering any definition of species concept. We cannot even use phylogenetic principles, for the species may have arisen from crossings similar to those described and shown diagrammatically in fig. 1, and they have probably collected their genes from very different sources. Phylogeny in the *Melanium* section shall probably not be illustrated in the manner of a branching tree, but sooner as an intricate network.

MÜNTZING's very interesting synthetisation of *Galeopsis Tetrahit* from the *pubescens* × *speciosa* cross (MÜNTZING 1930) strikingly shows how prudent we have to be as regards phylogenetic relationship. The artificial *Tetrahit* arose from a group, which should be supposed to be a phylogenetic different one, as its two parent species are neither capable of crossing with spontaneous *Tetrahit* nor with the artificial one, while artificial *Tetrahit* crosses with as well the spontaneous *Tetrahit* as its near relative *bifida*; in its chromosome number ($n = 16$) the artificial *Tetrahit* corresponds with the two latter and not with its own parents ($n = 8$). Crossing possibilities do not necessarily imply phylogenetic relationship but probably only a certain kind of genic affinity; the morphological relationship dealt with in taxonomy is probably for the main part contingent upon genic conformity, not always on a joint phylogenic development.

The *Melanium* section and in first line the *Tricolores* subsection

gives the impression of being a young group still in full development, as its species are not yet delimited by the boundaries of intersterility.

The present paper in itself has the character of being a summary of the investigations. A real summary implying all the facts will, therefore, not be given, but the results of the individual crossings are summarized in tables 1 and 2 (pp. 224—227). Apart from the main conclusions drawn on the foregoing pages some concrete and general results will here briefly be abstracted:

(1). *Viola diffusa* is most naturally classified in the *Melanium* section, not as before in the *Nomimium* section (p. 235).

(2). The Botanic Garden type of *V. Battandierii* shows an oscillating number of chromosomes, n = abt. 26—30 (p. 233).

(3). The chromosome number in itself does not determine the morphological type; dominance is contingent upon the action of the genes, not upon the chromosome numbers of the parents (p. 236).

(4). Almost all the *Melanium* hybrids show variable conjugation of their chromosomes. Many of them show polysomic association of the chromosomes, although the parents have normal disomic conjugation.

(5). The species of the *Melanium* section constitute a complete series of transitions as regards intersterility, morphological differences and conjugation of chromosomes in their hybrids. It is therefore impossible to draw any sharp line between differences of specific and of subspecific order (instances: Cross 4 and Cross 9).

(6). Types belonging to one and the same species, morphologically spoken, show the heaviest degree of intersterility in any *Melanium* hybrid (Cross 19, p. 259 and 287).

(7). There is no absolute coincidence between the degree of sterility and the amount of chromosomal conjugation.

(8). The specific significant differences are determined by Mendelian genes, but due to chromosomal irregularities they do not always segregate in regular Mendelian ratios. Even characters distinguishing taxonomically larger groups segregate.

(9). The wild growing species are characterised by a very intricate and complicated cooperation of genes (see for instance the inheritance of flower colours, pp. 239—242, table 4).

(10). The genetical analysis shows some accordance between *alpestris* and *arvensis* (Cross 8, p. 276).

(11). There is good accordance between the degree of polyploidy of the *Melanium* species and the degree of polymery (number of multi-

valent genes), which they show as to the basal genes for anthocyanin, the *A*-genes (table 13, p. 292—293).

(12). Chromosomes from species, which do not cross directly, may be brought together through quadruple hybridisation (Cross 24, p. 288).

(13). In some hybrids the later generations tend to increase the chromosome number above the number of F_1 (often connected with doubling of some chromosomes); other hybrids tend to decrease the chromosome number as compared with F_1 (elimination of single chromosomes).

(14). New constant, very fertile and vigorous species can be isolated in the offspring of the specific hybrids. The following types were named or mentioned: *V. phæno-elegantula* (p. 265), *V. crassicaulis* (p. 270), *V. hyperchromatica* (p. 277), *V. petaloidea* and *V. velutina* (p. 279). They seem to be just as characteristic as many wild growing species and show similar pecularities. The species applied for the present crossings grow together in the same areas. Especially in the area of the Balcan Peninsula may such new species be formed.

(15). Single genes can be introduced from one species into another, irrespective of the difference in chromosome number. Thus was the gene *D* for dilute mauve flower colour introduced from *arvensis* into a 13-chromosome *tricolor* (p. 276—277, Cross 9).

(16). Crossing favours »segmental interchange» between non-homologous chromosomes through later generations, resulting in multivalent association of the chromosomes into long chains (p. 271, figs. 119, 121—122).

(17). In F_3 of Cross 7 was a plant segregated, which deviated from what is normal in *Viola* as to the shape of chromosomes in meiosis. The chromosomes were long as in certain *Compositæ* and *Gramineæ* (figs. 123—126, p. 272). An F_2 plant of Cross 13 suggested an aggregation of two chromosomes into one (figs. 136—138, p. 281).

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ÜBER DAS ENTSTEHEN EINES GANZ CINEREA-ÄHNLICHEN TYPUS AUS DEM BASTARD SALIX VIMINALIS × CAPREA

VON *NILS HERIBERT NILSSON*
. ALNARP, ÅKARP

BEI meiner Analyse von F_2 der Kreuzung *Salix viminalis* × *caprea* war es auffallend, dass ein Strauch erschien, der zwar intermediär war, aber mehr an einen gedachten Bastard *viminalis* × *cinerea* als *viminalis* × *caprea* erinnerte (HERIBERT NILSSON 1918, p. 54). Weiter fand ich in F_2 einen sehr extravaganten Typus, den ich mit der alten Gartenart *S. laurina* (J. E. SMITH) WILLD. habe identifizieren können, die von den Floristen allgemein als *S. cinerea* × *phylicifolia* betrachtet wird (HERIBERT NILSSON 1928). Schon hieraus geht hervor, dass Eigenschaften, die an *cinerea*-Merkmale taxonomisch stark erinnern, aus den Arten *caprea* und *viminalis* hervorgebracht werden können. Aus den Spaltungsverhältnissen habe ich auch geschlossen, dass *caprea* zwei Faktoren für Blattbreite hat, die aber eine verschiedene Formwirkung haben, indem der eine eiförmige, der andere verkehrt eiförmige Blätter erzeugt (HERIBERT NILSSON 1918, p. 73). Weil *S. caprea* eine Kompromissform der Wirkung dieser beiden Faktoren ist, erhält sie Blätter, die in der Mitte am breitesten sind. Bei Kreuzung mit anderen Arten, bei welchen diese beiden Faktoren fehlen, z. B. mit *viminalis*, müssen in F_2 Individuen hervorgehen, die nur den einen der erwähnten Formfaktoren haben. Ist dieser der Faktor, der die grösste Breite oberhalb der Mitte des Blattes bewirkt, müssen morphologisch gesehen *cinerea*-ähnliche Merkmale hervortreten, obgleich der gekreuzte Elter *caprea* ist. Ich habe diesen Faktor, weil er die *cinerea*-ähnlichen Blätter der *S. laurina* verursacht, den *laurina*-Faktor genannt.

Falls nun diese meine frühere Annahme der erblichen Konstitution der Blattform von *S. caprea* richtig ist, so wäre auch zu erwarten, dass extrem sehr *cinerea*-ähnliche Individuen aus der Kreuzung *viminalis* × *caprea* hervorgehen könnten, nämlich wenn der *laurina*-Faktor rein ausspaltete, ohne den anderen *caprea*-Faktor und ohne die speziellen Formfaktoren von *viminalis*.

Diese Erwartung hat sich auch in meinen weiteren Versuchen bestätigt. Im Jahre 1928 habe ich eine neue Nachkommenschaft des Bastardes *viminalis* × *caprea* aufgezogen, in der Absicht, neue Extrem-

typen oder vielleicht noch einmal *S. laurina* aus der Kreuzung erhalten zu können. Unter den 524 Individuen der F_2 trat eines auf, das schon im zweiten Jahr durch seinen extravaganten Typus von sämtlichen Geschwisterpflanzen abwich. Da ich anfangs vermutete, dass ich noch einmal den *laurina*-Typus erhalten hatte, wurde die Entwicklung dieser Pflanze schon vom zweiten Jahre an genau verfolgt.

Meine Aufmerksamkeit wurde auf diese Pflanze zuerst deshalb gerichtet, weil *ihr architektonischer Aufbau ein ganz anderer war als der sämtlicher Geschwisternachkommen. Während diese mit einem dominierenden Hauptstamm heranwuchsen, blieb dieser bei der betreffenden Pflanze, Nr. 1731, in dem Zuwachs zurück.* Basale Seitenstämme wuchsen aber kräftig heran. Die Aufnahme in Fig. 1 zeigt in der Mitte den kurzen Hauptstamm der zweijährigen Pflanze. Die Architektonik des Strauches in der Frühling des vierten Lebensjahres (1931) wird von der Fig. 2 veranschaulicht. Der Hauptstamm lebt noch und ist als ein winziges Zweiglein in der Mitte des Strauches zu sehen (mit x markiert). Die Seitenstämme gehen von der Basis dieses Zweigleins kandelaberförmig aus. Dieser Typus der Verzweigung war für den *laurina*-Typus charakteristisch (HERIBERT NILSSON 1918, p. 59). Da die Blätter der zweijährigen Pflanze auch eine von den Geschwisterpflanzen abweichende Behaarung hatten, indem die Haare lang wie *laurina*-Haare waren, lag die Vermutung nahe, dass diese Neukombination zum zweiten Male erschienen hatte.

Fig. 1. Einige Pflanzen des zweiten Entwicklungsjahres aus F_2 der Kreuzung *viminalis* × *caprea*. Links der *neocinerea*-Typus. Der kleine Hauptspross ist in der Mitte der Pflanze zu sehen, zwischen den schon sehr kräftigen Seitensprossen. Rechts einige Geschwisterpflanzen.

Die Entwicklung des Strauches das dritte Lebensjahr zeigte aber, dass er keine *laurina* sein könnte, sondern er entschleierte sich als einen sehr *cinerea*-ähnlichen Typus. Die dieses Jahr stattgefundene Blüte hat ausserdem gezeigt, dass er auch in dieser Hinsicht als eine unverkennbare *cinerea* betrachtet werden muss.

Der schon behandelte Verzweigungstypus ist nicht nur für *laurina* charakteristisch, sondern ist auch ein *cinerea*-Charakter. Diese Art ist nämlich durch erst anliegende, dann aufsteigende Zweige ausgezeichnet. Durch wiederholtes Niederliegen der sukzessiven unteren Seitenzweige erhält der freistehende, ältere Strauch oft einen mächtigen Umkreis; der Durchmesser kann 5 m oder sogar noch mehr werden. *Nr. 1731* ist ja noch jung, weshalb die Architektonik noch nicht definitiv beurteilbar ist; *der durch die aufsteigende Zweige kandelaberförmige Verzweigungstypus der cinerea ist jedenfalls sehr ausgeprägt.* Die Äste scheinen vielleicht für eine *cinerea* ziemlich lang und schlank (vgl. Fig. 2). Der Strauch weist in dieser Hinsicht noch Merkmale von *viminalis* auf, wie übrigens auch der *laurina*-Typus dies tut (HERIBERT NILSSON 1928, p. 53). Auch die Farbe und die Behaarung der jüngeren Zweige deuten *viminalis*-Charaktere an, indem der Haarfilz nicht so dicht wie bei *cinerea* ist, sondern die Zweige dünnbehaart oder verkahlend filzig sind, und weil sie eine gelbliche statt graue Farbe haben. Die älteren Zweige haben indessen die rauhe, graue *cinera*-Farbe.

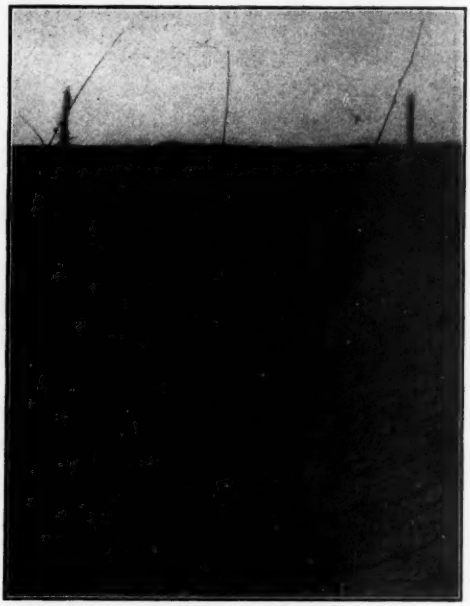

Fig. 2. Der *neocinerea*-Strauch vor dem Schossen das vierte Entwicklungsjahr. Der stark zurückgebliebene Hauptspross (durch × markiert) und die kandelaberförmige Verzweigung des Strauches treten hier deutlich hervor.

Die Knospen der Nr. 1731 waren von der *cinerea*-Form (HERIBERT NILSSON 1908, p. 202, Fig. 20 und p. 207), also dick, stumpf zugespitzt, gelb oder gelbgrau, behaart, wenn auch nicht so dicht graufilzig wie oft bei *cinerea*. An der Basis waren sie gewöhnlich mehr oder minder intensiv rötlich gefärbt. Dieser Charakter, den ich früher auch bei *laurina* gefunden habe, scheint aus *viminalis* abzustammen (HERIBERT

Fig. 3. Blatttypus der *neocinerea*, links Sommerblätter, rechts Herbstblätter.

Fig. 4. Ähren der *neocinerea*, rechts neuausgeschlagen, links mit fast reifen Kapseln.

NILSSON 1928, p. 54). *Sowohl in bezug auf die Form als die Behaarung sind die Knospen der Nr. 1731 sehr cinerea-ähnlich.*

Der Blatttypus des Strauches erinnert auch sehr auffallend an den der *cinerea,* was ja vielleicht besser als durch eine lange Beschreibung aus den Fig. 3 und 4 hervorgeht. In Fig. 3 gehören die beiden Zweigstücken zu demselben Zweig. Der kleinblättrige Teil ist der untere. Der Strauch war dann noch klein, nur zirka $^1/_2$ m hoch. Die Blätter erinnern noch an die Vorsommerblätter der *laurina,* sind aber etwas länger und schmäler, sowie auch länger zugespitzt. Die späteren, grösseren Herbstblätter sind aber so *cinerea*-ähnlich, *dass niemand den Verdacht hegen könnte, dass hier keine reine cinerea vorläge.* Die Blätter sind verkehrt eiförmig mit einer recht starken Neigung zu lanzettlich, kurz und plötzlich zugespitzt mit etwas umgedrehter Spitze, ausgebissen gesägt oder gekerbt, zum Grunde verschmälert, an der Basis spitz. Die Farbe ist oberseits graugrün oder schmutzig grün, trüb, unterseits bläulich graugrün. Alle diese Merkmale sind ganz typische *cinerea*-Eigenschaften.

Ebenso weicht die Behaarung nicht von dem *cinerea*-Typus ab. Jene wich schon in dem zweiten Entwicklungsjahr von der Mehrzahl der F_2-Sträucher dadurch ab, dass die Haare lang waren, nicht kurze Silberhaare wie bei dem F_1-Bastard. Auch hatten die Haare in diesem Stadium eine Tendenz zu Vorwärtsrichtung wie bei *laurina.* Im dritten Entwicklungsjahr war aber die Form und Richtung der Haare vom Typus der *Cinerascentes,* welcher Gruppe die Stammart *caprea* und ausserdem *cinerea* und *aurita* angehören. Charakteristisch für diesen Typus ist, dass die recht langen Haare nicht gerade, sondern etwas gekräuselt oder bogig gekrümmt sind; sie sind also nicht anliegend. Weiter ist ihre Richtung sehr typisch, indem sie allseitswendig sind. Dieser Typus der Behaarung war nun an den Herbstsprossen, also an den grossen Blättern des Zweigstückes der Fig. 3 sehr ausgeprägt. Die mehr *laurina*-ähnliche Behaarung von längeren, mehr vorwärtsgerichteten und anliegenden Haaren der ganz jungen Pflanze war deshalb nur ein Übergangsstadium. Nr. 1731 stimmt also als herangewachsener Strauch mit der Mutterart *caprea* in bezug auf den Typus der Behaarung vollkommen überein. Dass dieser aus dem Bastard *viminalis* × *caprea* rein herausspalten kann, habe ich früher gefunden, indem ein auch in bezug auf die Behaarung ganz reiner *caprea*-Strauch, Nr. 527, in der F_2-Nachkommenschaft dieses Bastards aus dem Jahre 1911 auftrat (HERIBERT NILSSON 1918, p. 62). Die Dichte der Behaarung, die eine der Artdifferenzen zwischen *caprea* und *cinerea* bildet, indem die jungen Blätter der ersten Art unterseits gewöhnlich

einen dichten weissglänzenden Haarfilz haben, die der letzteren nur zerstreute, wenn auch oft dicht sitzende, graue Haare, stimmt aber bei Nr. 1731 mit *cinerea*, nicht mit *caprea* überein. *Sowohl in bezug auf die Blattform als den Behaarungstypus stimmt also die extravagante Pflanze Nr. 1731 vollkommen mit S. cinerea überein.*

Die Blütezeit war bei Nr. 1731 dieselbe wie die der *cinerea*, also etwas später als bei *caprea*. Die Entwicklung der Kätzchen war wie bei den Eltern und wie die Fig. 4 zeigt vorläufig.

Auch die Ähren hatten eine auffallende *cinerea*-Prägung. Sie waren wie durchschnittlich bei dieser Art recht lang, die ausgewachsenen auch ziemlich locker, während *caprea* grössere, dickere, dichtblütigere Ähren hat. Der Stiel des Fruchtknotens war lang und wie bei *cinerea* ungefähr dreimal so lang als das Nektarium, was eine intermediäre Länge zwischen den Eltern repräsentiert, von welchen *caprea* sehr langen, *viminalis* sehr kurzen Fruchtknotenstiel hat. Die Form und Grösse des Fruchtknotens war die der *cinerea*, sowie auch die Behaarung. Wie bei dieser Art war der Griffel kurz, nur $^1/_8$—$^1/_{10}$ des ausgewachsenen Fruchtknotens (ohne Stiel). Die Narben waren noch kurzer als der Griffel, geteilt, abstehend. Der Strauch stimmte auch in diesen letzteren Charakteren mit *cinerea* überein und wich von den Eltern ab, von welchen *viminalis* sehr lange Narben hat, *caprea* zwar kurze, aber nicht abstehende, sondern zusammenneigende. *Kein einziger wichtigerer Charakter der nicht cinerea-ähnlich war, war bei den Ähren der Nr. 1731 zu entdecken.*

Als ein Merkmal ersten Ranges haben einige Forscher die Eigenschaft des gestriemten Holzes der *cinerea* betrachtet (vgl. HERIBERT NILSSON 1928, p. 60 u. f.). Man wollte vielleicht von floristischem Gesichtspunkte als ganz entscheidend für die Deutung der Artzugehörigkeit von Nr. 1731 betrachten, ob sie Holzstriemen hat oder nicht. *In der Tat zeigt dieser cinerea-ähnliche Typus auch das als besonders wichtig angesehene Merkmal der Holzstriemen auf.* Fig. 5 veranschaulicht sehr gut diesen Charakter. Das Holzstück stammt aus einem jungen, nur zweijährigen Zweig, aber die Striemen sind schon deutlich, wenn auch noch ziemlich kurz.

In bezug auf alle wichtigen taxonomischen Merkmale stimmt also Nr. 1731 mit S. cinerea überein. Sie kann also mit Recht S. neocinerea genannt werden. Denn in der Natur gefunden, wäre sie ohne geringsten Zweifel in S. cinerea eingereiht worden.

Nach den Untersuchungen von BLACKBURN und HARRISON (1924) weicht indessen *cinerea* von *caprea* und *viminalis* auch zytologisch ab,

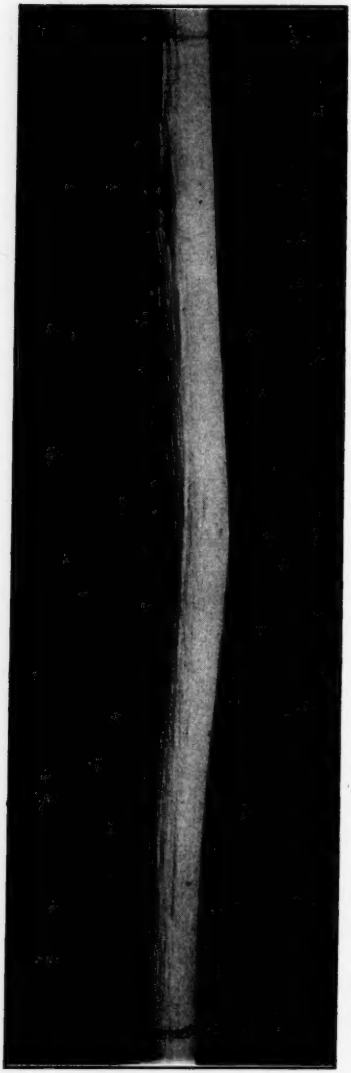

Fig. 5. Geschältes Zweigstück von *neocinerea*, die Holzstriemen zeigend (vergrössert).

indem jene Art tetraploid ist, also 38 n-Chromosomen statt 19 wie die letzteren hat. Eine vorläufige Untersuchung, die ich von Wurzelspitzen nach der Methode von KATTERMANN (1930) vorgenommen habe, zeigt, dass die Chromosomenzahl mindestens triploid ist. In einer deutlichen Metaphasenplatte fand ich 57 Chromosomen und in anderen, wo die Rechnung nicht exakt durchgeführt werden konnte, schien jedenfalls kaum die tetraploide Zahl 76 vorzuliegen. Anderseits war die Fertilität bei Kreuzung mit meinem in den Experimenten verwendeten ♂-Strauch der *cinerea* recht gut, was vielleicht eher auf Tetraploidie deuten sollte. Ich habe die Untersuchung nicht augenblicklich fortsetzen können, weil ich noch nicht mehr Material von dem *neocinerea*-Strauch habe opfern wollen. Fixierungen von den Ähren sind von Dozent HÅKANSSON vorgenommen, der über die embryologischen und zytologischen Verhältnisse des Strauches berichten wird. Dass *neocinerea jedenfalls polyploid, mindestens triploid ist, und also auch in dieser Hinsicht von den Eltern* abweicht und sich *cinerea* nähert, ist sicher.

Dass ein der Art *S. cinerea* L. ganz ähnlicher Strauch aus den Arten *S. caprea* L. und *S. viminalis* L. entsteht, kann ja paradoxal erscheinen. Früher habe ich indessen aus derselben Kreuzung die ganz eigentümliche Gartenart *S. laurina* (SM.) WILLD. erhalten, für deren Eltern man früher *S. cinerea* L. und *S. phylicifolia* L. (zwar unter zehn anderen!) angenommen hat (HERIBERT NILSSON 1928).

Auch dieser Typus hat, ebenso wie der *neocinerea*-Typus, sowohl gestriemten Holz wie eine überzählige Chromosomenzahl, die nach den Untersuchungen von HÅKANSSON (1929) hypertetraploid ist. *S. laurina* ist indessen ein ganz ausgeprägter Sondertypus, während *neocinerea* taxonomisch mit *cinerea* ganz übereinstimmt.

Es kann vielleicht sehr unwahrscheinlich erscheinen, dass die Art *cinerea* ein Kreuzungsprodukt sein sollte. Dass dieser Typus aus den Arten *caprea* und *viminalis* synthetisiert werden kann, ist indessen durch den Experimentalversuch gezeigt worden. Man fragt sich dann nur, ob wirklich die Art *cinerea* als eine Kreuzungsart angesehen werden kann?

Eine derartige Annahme scheint mir nicht unwahrscheinlich. Schon rein morphologisch liegt der *cinerea*-Typus in bezug auf mehrere Eigenschaften zwischen *caprea* und *viminalis*, z. B. Blattgrösse und Blattbreite, Blütezeit, Kapselgrösse, Länge des Kapselstiels u. s. w. Die Behaarung der Zweige und Knospen ist kein morphologisch neuer Charakter, denn dieselbe Behaarung, nämlich einen dunklen Haarfilz, hat auch *viminalis*. Auch die Striemen des Holzes kommen bei *viminalis* vor, zuweilen sogar bei *caprea*, sie sind nur bei *cinerea* verlängert und verstärkt. Als ein für *cinerea* ganz besonderes Merkmal könnte vielleicht die Blattform angesehen werden, indem die Blätter *oberhalb der Mitte* die grösste Breite haben, verkehrt eiförmig sind, während die Blätter der *caprea an der Mitte*, die der *viminalis* am Grunde am breitesten sind. Wie ich oben auseinandergesetzt habe (p. 309), ist indessen auch diese Eigenschaft aus *caprea* zu erklären.

Eine Voraussetzung für die Bildung der *neocinerea*-Kombination ist natürlich, dass die Elternarten zusammen wachsen und sich gegenseitig kreuzen können. Der Bastard *viminalis* × *caprea* ist fertil und gar nicht selten (HERIBERT NILSSON 1928, p. 77). Die Voraussetzung für das Entstehen des *neocinerea*-Typus ist damit gegeben. *Durch die polyphyletische Entstehung des Typus in verschiedenen Teilen des grossen Verbreitungsgebietes der Stammarten über ganz Europa sowie West- und Nordasien ist das Konstituieren der polymorphen Art cinerea gegeben.* Ein Durchkreuzen mit den Stammarten ist durch die Sterilität verhindert, wie meine Versuche zeigen. *Weder bei Kreuzung der neocinerea mit caprea noch mit viminalis wurde Samen erhalten,* sondern die Kapseln waren ganz leer. *S. cinerea* gibt bei Kreuzung mit diesen Arten auch nur vereinzelte Samen (was ich vielleicht auch von *neocinerea* bei der Kreuzung in grösserem Massstabe erhalten hätte). Auch die erhaltenen, seltenen F_1-Bastarde *cinerea* × *caprea* und *cinerea*

× *viminalis* waren stark steril. Von *cinerea* × *caprea* habe ich bei der Rückkreuzung mit den Eltern niemals Samen erhalten, von *cinerea* × *viminalis* nur sehr vereinzelt. Die Natur dieser Sterilität können erst weitere Versuche, experimentelle, embryologische und zytologische, entschleiern. Tri- und tetraploiden Formen sind ja gewöhnlich mit der Stammform gekreuzt sehr steril.

In dieser Synthese einer dritten Art aus zwei anderen — das zweite Beispiel in der Gattung *Salix*, indem ich früher die Synthese der *S. laurina* erzielt habe — könnte man wohl auch das Herstellen einer ausgeprägten und fertilen neuen Art von evolutionärem Wert erblicken. Denn *cinerea* ist ja eine sehr verbreitete und vitale Art. Ob sie aber im Kampfe ums Dasein mit den Eltern erfolgreich konkurrieren kann, ist jedenfalls zweifelhaft. Durch ökologische Untersuchungen im südlichen Schweden über die Verbreitung der Arten *caprea*, *aurita* und *cinerea* habe ich zeigen können, dass die beiden ersteren Arten in einer natürlichen, unbeeinflussten Vegetation eingehen können, während das Auftreten von *cinerea* eng mit den Kulturbedingungen zusammenhört (HERIBERT NILSSON 1930, p. 141). Die Art sollte den von RIKLI (1903) aufgestellten Apofyten angehören, womit er Pflanzen der natürlichen Vegetation meint, die sich an Kulturboden ansiedeln können. Die Verbreitung der *cinerea* spricht deshalb dafür, dass sie sich an natürlichen Lokalen entweder nicht leicht ansetzen kann oder von der Mutterart *caprea* und von *aurita* verdrängt wird. *S. cinerea* ist deshalb eine mit der Kultur zum Verschwinden verurteilte Art, womit auch ihr Wert in der natürlichen Auslese fraglich wird.

Eine interessante Parallele zu dieser Erscheinung bietet das Konstatieren von MÜNTZING (1930), dass er durch Kreuzung der *Galeopis*-Arten *pubescens* und *speciosa*, die diploid sind, die tetraploide Spezies *Tetrahit* herstellen können hat. Auch in diesem Falle ist die synthetische Art die wohl ausgeprägtest an den Kulturpflanzen gebundene von den erwähnten drei, die indessen alle zu dieser Gruppe zu rechnen sind.

Die bis jetzt hergestellten alten systematischen Arten (*Galeopsis Tetrahit* und *Salix cinerea*) gehören also alle beide den ökologischen Typen an, die RIKLI Anthropochoren genannt hat, also denjenigen, die nur in einer oder anderen Hinsicht aus der Gnade des Menschen existenzfähig sind. Sie sind also, von dem Gesichtspunkte der Selektion, ebensowenig wie die wahren Kulturarten in einem natürlichen Bestand existenzfähig. Sie erinnern in dieser Hinsicht an gewisse polyploide Mutanten der Zierpflanzen, die zwar unter der Pflege des Menschen vorzüglich gedeihen, die aber in der Natur bald ausgemerzt werden

würden. So ist z. B. *Oenothera gigas* eine in der Kultur durch ihre Stärke und ihren Samenreichtum ganz imponierende neue Art. In dem Felde bei Hilversum wurde sie aber ebensowenig wie die anderen, schwächeren Mutanten von DE VRIES (1901, p. 215) in den blühenden Beständen der Stammart gefunden. Es ist ja möglich, dass der Mensch durch den Anbau einer ganz besonderen Kulturpflanze die Verbreitung von *O. gigas* befördern könnte. Da dies augenblicklich nicht der Fall ist, ist sie auch von der direkten Pflege des Menschen abhängig. *Galeopsis Tetrahit* und *Salix cinerea* brauchen zwar keine direkte Pflege für ihre Existenz. Hält aber nicht der Mensch für *S. cinerea* offenen, gedüngten Boden als ökologisches Substrat, und baut er nicht für *G. Tetrahit* als ökologisches Medium die Getreidearten, würden auch diese Arten gewiss bald verschwinden.

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SIND DIE INDUZIERTEN MUTANTEN NUR SELEKTIVE ERSCHEINUNGEN?

VON *NILS HERIBERT NILSSON*
ALNARP, ÅKARP

WÄHREND der letzten Jahre hat man viele experimentelle Versuche ausgeführt, speziell mit *Drosophila,* die zeigen sollten, dass man durch äussere Faktoren eine Mutabilität hervorrufen kann. Man hat dabei verschiedene Agentien benutzt, vor allem veränderte Temperatur und Bestrahlung mit Radium- oder Röntgenstrahlen. MULLER, der derartige Versuche in grossem Massstabe ausgeführt hat, hat in dieser Weise die Mutabilität auf das 150-fache vermehren können, und ähnliche Resultate liegen von mehreren Forschern vor [Literatur bei MULLER (1928 b), TIMOFÉEFF-RESSOVSKY (1929) und JOLLOS (1930)]. An der *Tatsache* ist also offenbar nicht zu zweifeln.

Ob aber die *Deutung* dieser Erscheinung auch die richtige ist, nämlich dass man eine Mutabilität induziert hat, scheint mir gar nicht sichergestellt. Man erhält nämlich diese erhöhte Mutabilität nur in Kulturen, wo eine ganz katastrophale Sterblichkeit stattfindet. Je grössere Sterblichkeit, desto grössere Mutabilität! Dies deutet man so, dass die Mutabilität mit der Stärke des induzierenden Agens proportional ist, was man ja auch durch Kurven leicht veranschaulichen kann und auch tut. Es liegt aber auch eine andere Erklärung dieser Erscheinung nahe, auf die keiner gedacht zu haben scheint, nämlich dass diese extraordinäre Sterblichkeit selektiv auf die in dem Prozess beteiligten Genotypen gewirkt haben kann.

Auch diese Frage kann ja indessen experimentell gelöst werden. Ich habe deshalb mit einigen Mutanten der *Drosophila melanogaster,* die ich in Kultur habe, einige Versuche angestellt, um die Vitalität der Stammart und dieser Mutanten bei den für das Leben kritischen Temperaturen, denen die Tiere beim Mutationsexperiment ausgesetzt werden, zu prüfen. Mein Material stammt aus den Kulturen von MOHR, der es von MORGAN erhalten hat. Der hier benutzte Stamm war ausser der Wildform die *yw*-Mutante (gelbe Körperfarbe, weisse Augen), weil diese Eigenschaften, speziell die Weissäugigkeit, unter den öftest auftretenden Mutanteneigenschaften sind. Ausserdem scheint die Vita-

lität der *yw*-Tiere in der hier in Frage kommenden Periode eine sehr gute zu sein, wie meine Kontrollexperimente gezeigt haben.

Nach einigen orientierenden Vorversuchen wurden drei Versuchsserien durchgeführt. Die Fliegen wurden teils in Glasröhren (15 cm lang und 3 cm weit), teils in Milchflaschen von 200 cm^3 aufgezogen; Kultursubstrat war Bananenagar. In den Rohren wurden die Kulturen mit 5 ♀ und 5 ♂, in den Flaschen mit 10 ♀ und 10 ♂ angesetzt. Die Tiere wurden nicht entfernt, sondern setzten fort, Eier abzulegen, bis sie bei der Hitzebehandlung abstarben. Hierdurch beabsichtigte ich, auch die Sterblichkeit bei verschiedenem Alter der Larven zu prüfen, was ja auch für die hier aufgenommene Frage sehr wichtig ist.

In der ersten Serie wurden 4 Rohre mit dem Normaltypus (+) und 4 mit dem *yw*-Typus bei einer Temperatur von 36—36,5° C geprüft. Die ältesten Larven waren 6 Tage alt, aber gleichzeitig kamen alle Stadien bis zu den Eiern vor. Die eine Hälfte der Rohre wurde 24 Stunden der Hitzebehandlung ausgesetzt, die andere 48 Stunden. Das Resultat wird von der Tabelle 1 veranschaulicht.

TABELLE 1. *Die Kulturen wurden d.* $^{14}/_{3}$ *angesetzt, d.* $^{20}/_{3}$ *der Hitze ausgesetzt.*

Typus	Behandlung in Stunden	Anzahl der geschlüpften Fliegen bei den verschiedenen Abrechnungsdaten					
		$^{29}/_{3}$			$^{1}/_{4}$		
		♀	♂	Summe	♀	♂	Summe
+	24	12	12	24 | 40	27	23	50 | 78
+	»	13	3	16 |	14	14	28 |
yw	»	26	31	57 | 91	18	16	34 | 50
yw	»	15	19	34 |	10	6	16 |
+	48	—	—	0 | 0	—	—	0 | 0
+	»	—	—	0 |	—	—	0 |
yw	»	4	2	6 | 10	17	5	22 | 33
yw	»	4	—	4 |	6	5	11 |

Das Experiment war in der Hinsicht auffallend, dass nach 48-stündiger Behandlung keine einzige Fliege aus den Kulturen des Normaltypus ausschlüpfte, sondern nur die Rohre mit der Mutante *yw* Nachkommenschaft gaben. *Nur die Mutante, nicht aber die Stammform hat also die längere Induktionszeit überleben können.*

In den Kulturen nach 24-stündiger Induktion sind sowohl + als

yw geschlüpft, aber bei der ersten Abrechnung ($^{29}/_{3}$) sind die *yw*-Tiere mehr als doppelt so viel als die Normaltiere. Bei der letzten Abrechnung hat sich dieses Verhältnis zugunsten der +-Kulturen verschoben, was man vielleicht so deuten wollte, dass *yw* schneller als + schlüpfen. Wie die späteren Versuche zeigen, ist indessen die Ursache dieser Erscheinung eine andere. Die Abrechnung dieser Kulturen fand überhaupt zu spät statt, nämlich erst nach 15 Tagen, während das Schlüpfen schon nach 10—11 Tagen anfing. Die ersten Momente des Prozesses wurden deshalb verschleiert. Die späteren Versuche hatten also das Ziel, auch das Zunehmen der +-Tiere aufzuklären.

Ein zweiter Versuch wird in der Tabelle 2 demonstriert. Die Versuchsanordnungen waren dieselben wie in dem oben erwähnten. Nebst Rohren wurden hier auch Flaschen benutzt. Sowohl 24- als 48-stündiger Induktionszeit wurde verwendet.

TABELLE 2. *Die Kulturen wurden d.* $^{4}/_{4}$ *angesetzt, d.* $^{10}/_{4}$ *der Hitze ausgesetzt.*

Typus	Behandlung in Stunden	Art des Kulturgefässes	Anzahl der geschlüpften Fliegen bei den verschiedenen Abrechnungsdaten												Summa summarum sämtlicher Abrechnungsdaten	
			$^{15}/_{4}$			$^{17}/_{4}$			$^{20}/_{4}$			$^{25}/_{4}$				
			♀	♂	Summe	♀	♂	Summe	♀	♂	Summe	♀	♂	Summe		
+	24	Rohr	—	—	0	—	—	0	—	—	0	—	—	0	0	
yw	»	»	4	2	6	5	4	9	15	23	38	16	7	23	76	
+	48	Rohr	—	—	0	—	—	0	—	—	0	—	—	0	0	
+	»	»	—	—	0	—	—	0	11	3	14	26	15	41	55	55
+	»	»	—	—	0	—	—	0	—	—	0	—	—	0	0	
+	»	Flasche	—	—	0	—	—	0	—	—	0	—	—	0	0	
yw	»	Rohr	—	—	0	—	—	0	—	—	0	—	—	0	0	
yw	»	»	—	—	0	—	—	0	7	6	13	—	1	1	14	128
yw	»	»	—	—	0	—	—	0	—	—	0	—	—	0	0	
yw	»	Flasche	—	—	0	3	3	6	58	47	105	2	1	3	114	

Aus der Tabelle 2 geht hervor, dass die *yw*-Kulturen sowohl in bezug auf die Anzahl, in der die Tiere überhaupt die Hitze überlebt haben, als in bezug auf den Prozentsatz ausgeschlüpfter Tiere den Normalkulturen überlegen sind. Die Summe erhaltener + ist nur 55, während sie für *yw* 204 ist. Nur 21 % sämtlicher ausgeschlüpfter Tiere sind also von dem Typus der Stammart, während 79 % den

Typus der *yw*-Mutante repräsentieren. Dass also diese durchschnittlich, d. h. ohne Berücksichtigung des Alters der Larven, die extreme Hitze besser als die Wildform überlebt hat, ist ganz offenbar.

Für die hier aufgenommene Frage ist indessen nicht nur diese durchschnittliche Temperaturresistenz von Bedeutung. Von noch grösserer Wichtigkeit ist die Resistenz während des Stadiums, wo der stärkste Mutationseffekt erhalten wird, nämlich für 5—6 Tage alte Larven. Diese sind in den ersten Schlüpfungstagen zu finden. Betrachtet man nun die Tabelle 2, ist es überraschend, dass man *während der ersten drei Tage nur yw-Tiere geschlüpft findet.* Die ersten zwei Abrechnungen zeigen nämlich zusammen 21 Fliegen, alle ohne Ausnahme *yw*. *In der Periode, die man als die für eine induzierte Mutabilität besonders geeignete gefunden hat, ist also jedenfalls die yw-Mutante gegen das Induktionsagens resistenter als ihre Stammart.*

In der Kultur, wo ein Schlüpfen von + überhaupt stattgefunden hat, treten die ersten Fliegen wenigstens drei Tage später als die ersten *yw* auf. Und erst zehn Tage nach der erwarteten Schlüpfung ($^{25}/_{4}$) haben sie die maximale Schlüpfung. Dies kann kaum anders gedeutet werden, als dass die jüngsten Larven von + die Hitze vertragen, während die ältesten zu Grunde gehen. Die *yw* scheinen ihre maximale Schlüpfung fünf Tage nach dem Erscheinen der ersten Fliegen gehabt zu haben, wie sämtliche Kulturen ganz übereinstimmend zeigen. Die Vitalitätskurve ist also für die Wildform stärker zugunsten der jüngeren Larven verschoben als für die *yw*-Mutante. Dass die Ursache dieser Differenz nicht darin liegt, dass die Zeit des Schlüpfens für die beiden betreffenden Formen erblich verschieden ist, zeigt ein Vergleichen von Kulturen unter normalen Bedingungen, wo die ersten Fliegen gleichzeitig erscheinen.

Ein Verifizieren dieser Resultate erhielt ich noch in einer dritten Kultur, deren Resultate ich in Tab. 3 zusammengestellt habe.

Dieser Versuch ist ja eine ganz ideale Repetierung des vorigen (Tab. 2). Gerade wie dort ist während der ganzen Abrechnungszeit ein grösserer Prozentsatz von *yw* als von + erhalten; gerade wie dort sind während der ersten drei Tage *nur yw*-Tiere geschlüpft. Sowohl die Kulturen in Rohren wie in Flaschen stimmen in dieser Hinsicht überein. In dem Versuch gingen auch Kulturen ein, die während 48 Stunden der Hitze ausgesetzt waren. Eine Schwankung der Temperatur bis auf 37° bei dem Ausnehmen der 24 Stunden behandelten Rohre hat aber ganz tötend gewirkt, denn in sämtlichen Kulturen

TABELLE 3. *Die Kulturen wurden d.* $^{8}/_{5}$ *angesetzt, d.* $^{15}/_{5}$ *der Hitze ausgesetzt.*

Typus	Behandlung in Stunden	Art des Kulturgefässes	Anzahl der geschlüpften Fliegen bei den verschiedenen Abrechnungsdaten												Summa summarum sämtlicher Abrechnungsdaten	
			$^{21}/_{5}$			$^{23}/_{5}$			$^{24}/_{5}$			$^{29}/_{5}$				
			♀	♂	Summe	♀	♂	Summe	♀	♂	Summe	♀	♂	Summe		
+	24	Rohr	—	—	0	—	—	0	7	1	8	21	30	51	59	
+	»	»	—	—	0	—	—	0	—	—	0	4	—	4	4	
+	»	»	—	—	0	—	—	0	—	—	0	1	—	1	1	171
+	»	»	—	—	0	—	—	0	—	—	0	—	—	0	0	
+	»	Flasche	—	—	0	—	—	0	1	—	1	59	48	107	107	
yw	»	Rohr	—	—	0	3	1	4	6	3	9	24	23	47	60	
yw	»	»	—	—	0	1	—	1	5	2	7	17	14	31	39	
yw	»	»	—	—	0	—	—	0	—	—	0	—	—	0	0	279
yw	»	»	—	—	0	2	2	4	—	—	0	18	17	35	39	
yw	»	Flasche	1	—	1	2	—	2	8	6	14	62	62	124	141	

(6 + und 6 *yw*, sowohl Rohren als Flaschen) fand kein Schlüpfen statt.

Die einzige Deutung, die man das Resultat der hier behandelten sehr übereinstimmenden Versuche geben kann, ist, dass der Mutantentypus yw bei der induzierten, kritischen Temperatur eine bessere Vitalität hat als die Wildform, die Mutterart. Dass der Prozentsatz dieser Mutante in Grosskulturen mit der Wildform bei Temperaturinduktion ansteigt, ist dann nicht eigentümlich, weil die seltenen Mutanten überleben, während die Stammart gerade bei der »geeigneten» Induktionstemperatur katastrophal abgetötet wird. Die Temperatur ist also nicht die Ursache der Mutabilität, sondern nur der geeignete Selektionsmoment für das bessere Überleben der yw-Form. Die scheinbar induzierte Mutabilität ist also nur eine selektive Erscheinung.

Da meine verwendete Mutantenform *yw* war, ist es auch zu erwarten, dass Mutanten von *y*- oder *w*-Typus, oder alle beide, in den ausgeführten Induktionsexperimenten unter den gewöhnlicheren sein sollten. Denn je grössere Vitalität eine Mutante bei der kritischen Wirkung des Agens hat, desto mehr muss ihr Prozentsatz in »le beau reste» ansteigen. Eine Übersicht der einschlägigen Literatur über induzierte Mutanten scheint auch zu zeigen, dass jedenfalls die Weissäugigkeit (*w*) eine der gewöhnlichsten Formen ist. Selbst habe

ich sie bei Kälteexperimenten, die auch mit + und *yw* ausgeführt wurden, einmal aus + erhalten. (Diese Experimente, die bei + 2,5° C ausgeführt wurden, deuten in dieselbe Richtung wie die Wärmeexperimente, sind aber noch zu fragmentärisch für ein Entscheiden.) In Induktionsexperimenten ist die *w*-Mutanten von folgenden Forschern und in folgender Anzahl Fälle gefunden:

MULLER	(1927, p. 259)	4 *w*-Individuen;
HANSON	(1928, » 361)	1 » » ;
»	(1929, » 284)	2 » » ;
GOLDSCHMIDT	(» , » 440, 441)	11 » » ;
TIMOFÉEFF-RESSOVSKY	(» , » 631)	2 *w*- (aus eosin) Individuen;
JOLLOS	(1931, » 173)	3 *w*-Individuen;
HERIBERT NILSSON	(oben, » 325)	1 » » .

Dass also *w*-Mutanten unter den nach Induktion häufigst auftretenden sind, geht aus fast allen mehr ausgedehnten Kulturen hervor. Seltener ist die *y*-Mutante, die in den eben erwähnten Versuchen nur von JOLLOS (1931, p. 173) einmal gefunden worden ist. Der Effekt in bezug auf die von mir angewendete *yw*-Doppelmutante muss wohl deshalb speziell von *w* verursacht sein.

Es kann ja eigentümlich erscheinen, dass die Mutanten von *Drosophila*, die als Imagines eine eher geringere Vitalität als die Mutterart haben, als Larven in bezug auf diese Eigenschaft die Wildform übertreffen. Es ist deshalb von grösstem Interesse, dass eine Pflanzenart, und gerade *Oenothera Lamarckiana,* deren Variabilitätserscheinungen in den letzten Jahren so oft mit denen der *Drosophila* verglichen worden sind, ganz parallele Erscheinungen aufweist. Falls man nämlich die Samen dieser Art bis an der Grenze der Vitalität aufbewahrt, die gewöhnlich bei 4—5 Jahren liegt, so erhält man ein ganz erstaunlich erhöhtes Mutationsprozent. Während dieses gewöhnlich 1—3 % ist, kann sie bis auf 100 % gesteigert werden, d. h. alle keimfähigen Samen geben Mutanten. Eine Zusammenstellung der Nachkommenschaften, die ich während mehrerer Jahre aus 4—6 Jahre altem Samen aufgezogen habe, gibt Tab. 4.

Aus der Tabelle ist ersichtlich, dass die Keimfähigkeit der Samen von *Oenothera Lamarckiana* schon nach 4 Jahren so stark herabgesetzt ist, dass einige Nachkommenschaften keine Pflanzen geben. Nach fünf Jahren sinkt sie noch mehr. Nach 6 Jahren erhält man nur aus-

TABELLE 4. *Resultat von dem Aussaat alten Samens der Oenothera Lamarckiana.*

Nr. und Anbaujahr der Stammpflanze	Aussaatjahr der Nachkommenschaft	Alter der Samen in Jahren	Anzahl Pflanzen des Muttertypus	Anzahl Pflanzen von Mutantentypus	Mutationsprozent
$^{20}/_{139}$ 1914.....	1918	4	2	—	0
$^{12}/_{1}$ 1914.....	1918	4	4	—	0
$^{31}/_{35}$ 1917.....	1921	4	—	—	—
$^{31}/_{4}$ 1917.....	1921	4	—	—	—
$^{32}/_{1}$ 1917.....	1921	4	1	3	75
$^{30}/_{33}$ 1917.....	1921	4	2	—	0
$^{31}/_{35}$ 1917.....	1922	5	—	1	100
$^{31}/_{4}$ 1917.....	1922	5	—	—	—
$^{32}/_{1}$ 1917.....	1922	5	—	1	100
$^{30}/_{38}$ 1917.....	1922	5	—	—	—
$^{7}/_{2}$ 1919.....	1923	4	—	—	—
$^{6}/_{2}$ 1922.....	1927	5	9	2	18
$^{8}/_{8}$ 1922.....	1927	5	5	2	29
$^{5}/_{2}$ 1923.....	1929	6	—	2	100
$^{5}/_{38}$ 1925.....	1930	5	19	1	5
$^{1}/_{14}$ 1926.....	1930	4	2	—	0
$^{6}/_{22}$ 1925.....	1930	5	1	1	50
$^{2}/_{10}$ 1926.....	1930	4	3	1	25
$^{8}/_{32}$ 1925.....	1930	5	1	2	33
$^{3}/_{12}$ 1926.....	1930	4	5	1	17
$^{9}/_{22}$ 1925.....	1930	5	—	—	—
Summe	—	—	54	17	24

nahmsweise Pflanzen. *Mit dem Alter des Samens, also mit der herabgesetzten Keimfähigkeit, geht das Ansteigen des Mutationsprozents ausgesprochen parallel.* Bei der einzigen Kultur aus sechsjährigem Samen ist es 100 % (nur Mutanten), bei den Kulturen, die nur 5 Nachkommen gegeben haben, wo also die Keimfähigkeit die niedrigste gewesen ist, erhält man den Prozentsatz 40 % (11 Mutanten auf 27 Pflanzen), für sämtliche Kulturen aus vier- bis sechsjährigem Samen ist es, wie die Tab. 4 direkt zeigt, 24 %, um in Kulturen aus vorjährigem Samen auf rund 3 % zu sinken (HERIBERT NILSSON 1915, p. 76). HUGO DE VRIES erwähnt auch eine Kultur aus Samen nach fünfjährigem Aufbewahren, die 40 % Mutanten, statt 5 % in Schwesterkulturen, gegeben hat (1901, p. 185). Der Samen der letzten fünf Kulturen war zwar

ebenso alt, aber die Keimfähigkeit der Samen war hier gut, während sie in der erstgenannten Kultur sehr schlecht war. Die erhöhte Mutabilität geht also auch in diesem Versuch mit dem schlechten Keimen parallel.

Dieser unwidersprechliche Zusammenhang zwischen erhöhter Mutabilität und herabgesetzter Keimungsenergie der Embryonen erinnert ja ganz auffallend an die Erscheinung der induzierten Mutabilität bei *Drosophila*. In beiden Fällen ist das gesteigerte Mutationsprozent von dem grösseren Absterben der jungen Zygoten (Larven, Embryonen) des Normaltypus als des Mutantentypus verursacht. Während man aber bei *Drosophila* selbst ein Agens in einem gewissen Stadium der Entwicklung des Tieres induziert und dadurch glaubt, Mutationen durch diesem Agens ausgelöst zu haben, so ist ja eine derartige Erklärung in bezug auf die erhöhte Mutabilität bei *Oenothera* nicht naheliegend. Denn hier wird keine Induktion vorgenommen, die Mutabilität ändert sich von selbst mit dem Herabgehen der Keimungsenergie der Samen. Es ist dann sehr naheliegend, diese Erscheinung durch ein selektives Absterben eines gewissen Zygotentypus zu erklären. *Es scheint mir aber mehr als wahrscheinlich zu sein, dass die beiden Prozesse ganz wesensgleiche selektive Erscheinungen sind.*

An Spekulationen für die Erklärung der induzierten Mutabilität hat es sonst nicht gefehlt. Die Effektivität der Röntgen- und Radiumstrahlen als Agentien hat gewisse Forscher (OLSON und LEWIS 1928, HALDANE 1929) auf den Gedanken geführt, dass radioaktive Prozesse der Erde oder kosmische Strahlen eine grosse Rolle für das normale Auslösen von Mutanten und damit für die Speziesbildung spielen könnten. Von diesen Überwiegungen veranlasst, haben BABCOCK und COLLINS (1929) Versuche angestellt, um die Mutabilität unter Bedingungen einer natürlichen Radioaktivität zu studieren. In einem Tunnel in San Francisko fanden sie eine doppelt so grosse Radioaktivität als in ihrem Laboratorium in Berkeley. Hier exponierte *Drosophila*-Fliegen zeigten eine etwas erhöhte Mutabilität. Ähnliche Versuche wurden fast gleichzeitig von HANSON und HEYS vorgenommen. In einer verlassenen Grube im westlichen Colorado fanden sie eine geeignete Lokalität, indem die Ionisierung der Luft dort 0,39 mal intensiver war als von ein mg Radium, wenn die Strahlen durch ein 0,156 Zoll dickes Bleifilter passiert hatten. *Drosophila*-Kulturen wurden hier für 140 Stunden der natürlichen Radiumbestrahlung ausgesetzt. Keine statistisch signifikative Differenz der Mutabilität gegen den Kontrollkulturen wurde indessen erreicht, ebensowenig wie in den oben erwähn-

ten Versuchen. Vielleicht eilen diese Versuche wie das durchgehende Pferd der ruhigen Wahrheit voraus!

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THE GEOGRAPHICAL DISTRIBUTION OF THE ALPINE ECOTYPE OF SOME EURASIATIC PLANTS

BY *GÖTE TURESSON*
LUND

AS is well known, numerous plant species ascend from the lowland region to high alpine levels. In the latter situations these species, as far as we know at present, are as a rule represented by hereditary, alpine ecotypes (TURESSON 1925 and 1930, POPLAWSKA 1930). On the basis of the assumption that the various biotypes composing such a species were uniformly spread, we should expect to find similar or closely related alpine ecotypes in the different alpine regions lying within the geographical distribution area of that species. To judge from the distribution in Europe of the subalpine ecotype, such a uniform biotype-spread would seem to prevail in species like *Solidago virgaurea* L., *Melandrium rubrum* (WEIG.) GARCKE, and *Myosotis silvatica* HOFFM. The subalpine ecotype of these species, growing in the Scandinavian mountain regions, shows strong resemblance to the corresponding type growing in the Alps, as I have pointed out some years ago (TURESSON 1925). A similar study of the distribution in Europe of the alpine ecotype shows that in some species this type may be present in the Scandinavian mountains, while it is altogether absent in the Alps, and vice versa. The former condition is met with in for instance *Melandrium rubrum* and *Ranunculus acer* L., which species — although very common in the subalpine region of the Alps — do not possess any alpine ecotype in the Alp range, and do not here enter *regio alpina* except in particularly sheltered places (cf. TURESSON 1925). Species like *Silene venosa* (GIL.) ASCHERS. and *Trifolium pratense* L., on the other hand, illustrate the reverse condition; although common in lowland regions of Scandinavia, they do not possess any alpine ecotypes in the North as is the case in the Alp range. Species of the two last mentioned kinds, to conclude from the assumption made, would indicate an irregular and unsymmetrical distribution of their constituent biotypes.

It is the object of the present paper to give further evidence on the question of the distribution of the alpine ecotype in its relation

to the biotype-composition of the species. During my journey in the Altaian mountains in Southern Siberia in 1927, new material suitable for a profitable discussion of this problem was obtained. This transplant material, as well as the comparative material previously brought home from various European localities, was grown at Åkarp, where also the seedling generations have been raised. The treatment of the material and the methods used in obtaining the data, recorded in the tables, have already been described in a paper of 1930 (TURESSON 1930). The measurements were all made in 1930, and the species population principally investigated refer to *Solidago virgaurea* L., *Bupleurum longifolium* L., and *Polygonum bistorta* L.

I. SOLIDAGO VIRGAUREA L.

The alpine ecotype from Scandinavia has been described in a previous publication (TURESSON 1925), where also subalpine and lowland ecotypes of the species are analysed. The former is a low growing type and starts its flowering period at Åkarp in the first part of June, while the tall growing type from South Sweden starts flowering in the latter part of July. In a subsequent paper (TURESSON 1930) much new material from various parts of Europe, including several seedling generations, have been analysed. Now it is a remarkable fact that no alpine ecotype has been found to occur in the Alps or in the Carpathians, although numerous series from these regions have been investigated. In the Bavarian and in the Austrian Alps, as well as in the Tatra mountains, scattered individuals of the species are found in sheltered places above the tree limit, but these individuals, upon cultivation, have all been found to represent much dwarfed modifications of the subalpine ecotype. It should be noted that no marked differences seem to exist between the subalpine ecotype of these regions and the Scandinavian subalpine type.

When visiting the East-Altaian mountain range, I was much struck by the frequent occurrence of certain species above the tree limit, which in Europe usually are confined to the subalpine region. On the mountains lying east of Lake Teletskoje, between the Gorbu mountain and the watershed separating the Ob from the Abakan river-systems, *Solidago virgaurea*, among others, extended invariably into the alpine region above the tree limit, which in this region is formed by *Pinus cembra* var. *sibirica* RUPR. with slight admixture of *Larix sibirica* LEDEB. On Mount Eschumo, where the tree limit runs at a

height of about 1800 m. above sea level, and where large collections of living alpine material were made, *Solidago virgaurea* grows on the rather dry mountain slopes together with such species as *Crepis chrysantha* (LEDEB.) TURCZAN., *Dracocephalum altaiense* LAXM., *Dryas octopetala* L., *Empetrum nigrum* L., *Gentiana algida* PALLAS, *Phyllodoce coerulea* (L.) BABINGT., and others. The plant attains here a height of 20—30 cm. and flowers freely in the latter part of July.

In order to compare the alpine material of the species with the lowland type, growing abundantly in the moist, wooded »taiga» of the region, collections were also made at Artebasch on the Northern shore of Lake Teletskoje (about 450 m. above sea level). The plant attains here a height of up to 1,75 m.

TABLE 1. *Height of the seedling generations of Solidago virgaurea.*

Source	n	Height (in cm.)		
		M	σ	m $\pm$
Finse	25	34,6	3,94	0,79
Eschumo	24	58,9	3,82	0,78
Artebasch	26	101,5	5,61	1,10

TABLE 2. *Differences in height between the different seedling generations of Solidago virgaurea together with their quotients D/m (in heavy types).*

	Artebasch	Eschumo	Finse
Artebasch	×	42,6 $\pm$ 1,35 **31,6**	66,9 $\pm$ 1,35 **49,6**
Eschumo	—	×	24,3 $\pm$ 1,11 **21,9**
Finse	—	—	×

From the group isolations made in these two series (from four plants in each group) full-grown seedling generations have now been raised. For the sake of comparison a seedling generation has also been raised from the Scandinavian alpine ecotype from Finse in Norway. The data on the height differences between these series are tabulated in tables 1 and 2. The alpine type from Eschumo is considerably taller than the alpine type from Finse, as seen from the

tables, while the lowland type from Artebasch corresponds as to height to the lowland types from South Europe (cf. TURESSON 1930). Fig. 1 brings out the remarkable difference in earliness between the two East-Altaian types. The alpine Eschumo type flowers about three

Fig. 1. *Solidago virgaurea.* Seedling plants raised from the Artebasch lowland series (to the left), and from the alpine Eschumo series (to the right). Plants potted immediately before photographing. Stick 30 cm. high. Photo 15. 6. 1930.

weeks earlier than the lowland type, as observed in 1929 and 1930. The Finse type, on the other hand, is somewhat earlier than the Eschumo type, while the Artebasch lowland type is slightly earlier than the lowland type of South Sweden.

II. BUPLEURUM LONGIFOLIUM L.

While this species in the Western parts of the Alp range is mostly confined to lower levels, it ascends towards the East into the subalpine

region. In the Carpathians it is found even in the upper part of the *Pinus montana*-belt. In 1924 a series was brought home from this level in the Hohe Tatra (above Tatra Füred), but no marked differences with regard to height and earliness were found between this series and cultivated transplant material of the species brought home from Schwarzwald. In the Altai region the species, represented by the var. *aureum* (FISCHER) H. WOLFF, grows abundantly on the foreland

TABLE 3. *Height and earliness of the seedling generations of Bupleurum longifolium.*

Source	n	Height (in cm.)			Earliness		
		M	σ	m ±	M	σ	m ±
Eschumo	27	40,3	2,98	0,57	+ 3,89	0,57	0,11
Artebasch	25	71,0	4,04	0,81	+ 1,30	0,32	0,06
Tatra	25	68,1	3,84	0,77	+ 1,26	0,36	0,07

TABLE 4. *Differences in height between the different seedling generations of Bupleurum longifolium together with their quotients D/m (in heavy types).*

	Artebasch	Tatra	Eschumo
Artebasch	×	2,9 ± 1,11 **2,6**	30,7 ± 0,99 **31,0**
Tatra	—	×	27,8 ± 0,96 **29,0**
Eschumo	—	—	×

TABLE 5. *Differences in earliness between the different seedling generations of Bupleurum longifolium together with their quotients D/m (in heavy types).*

	Eschumo	Artebasch	Tatra
Eschumo	×	2,59 ± 0,13 **19,9**	2,63 ± 0,13 **20,2**
Artebasch	—	×	0,04 ± 0,10 **0,4**
Tatra	—	—	×

as well as in the alpine region above the tree limit. On Mount Eschumo, as well as on other East-Altaian mountains, the plant is found associated with very much the same species as the alpine type of *Solidago virgaurea*. It attains here a height of about 15—25 cm. and flowers and fruits freely.

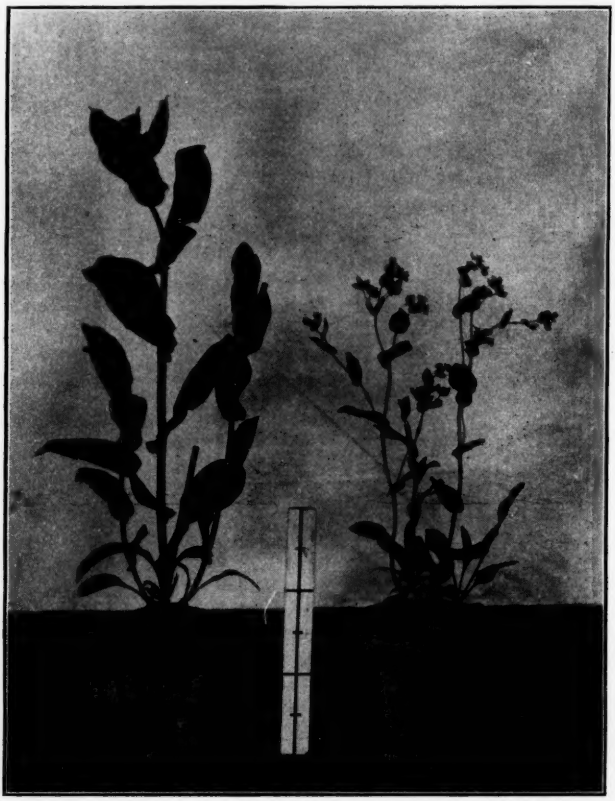

Fig. 2. *Bupleurum longifolium*. Seedling plants raised from the Artebasch lowland series (to the left), and from the Eschumo alpine series (to the right). Photo 3. 6. 1930.

The three seedling generations raised (from three group isolated transplants from each series) comprise the type from the alpine region on Mount Eschumo, the lowland type from the neighbourhood of Artebasch, and the Tatra series from the *Pinus montana*-level. Tables 3—5 contain the data on the differences in height and earliness between

the series. It is at once to be seen from the tables that the alpine Eschumo series is considerably lower in stature than the two other series. It is also clear that this series represents a much earlier type than the Artebasch and the Tatra series, which latter do not show any difference in earliness of statistical significance. Fig. 2 also illustrates the greater earliness of the alpine type in comparison with the Artebasch lowland type. The difference in time was found to be about two weeks in 1929 and 1930.

III. POLYGONUM BISTORTA L.

As is well known, this species is mainly confined to lower levels on the European mountains, reaching up in the subalpine region in some places. Ecotypic differences may exist between lowland and subalpine populations in Europe, but these must be very insignificant since no definite proof of the existence of such differences have been obtained in cultivated material. The material examined as to this point comprises series from the Bavarian Alps (lowland series from Garmisch-Partenkirchen and subalpine series from Schachen), as well as series from the Tatra mountains. In the Altai region *Polygonum bistorta* is a very common plant, covering large areas in the lowland, as well as in the alpine region above the tree limit. Along the shores of Lake Teletskoje, and on the flooded flats in the neighbourhood of Artebasch, the species is associated with such plants as *Achillea impatiens* L., *Aira cæspitosa* L., *Allium schoenoprasum* L. (cf. TURESSON 1931), *Caltha palustris* L., *Festuca rubra* L., *Polygonum alpinum* ALLIONI, and *Rumex aquaticus* L. The species reappears in the alpine region on Mount Eschumo and on other mountains in the Altai, where it forms extensive meadows together with the following characteristic associates: *Anemone narcissiflora* L., *Carex atrata* L., *Dracocephalum altaiense* LAXM., *Pedicularis uncinata* STEPH., *Salix Turczaninowii* LACKSCHEWITZ, *Sibbaldia procumbens* L., and *Trisetum subspicatum* (L.) P. DE BEAUV.

Seedling generations from group isolated transplants (three individuals in each group), originally collected in the subalpine region at Tatra Füred and at Schachen, have now been raised together with seedling generations of the Artebasch lowland series and the Eschumo alpine series. The data on the differences with regard to height and earliness found between the series are tabulated in tables 6—8. The Artebasch lowland type surpasses the rest considerably as to height,

TABLE 6. *Height and earliness of the seedling generations of Polygonum bistorta.*

Source	n	Height (in cm.)			Earliness		
		M	σ	m ±	M	σ	m ±
Tatra	23	98,4	6,71	1,40	+ 1,35	0,43	0,09
Schachen	27	95,6	6,05	1,16	+ 1,37	0,55	0,11
Artebasch	24	120,5	8,76	1,79	+ 1,83	0,49	0,10
Eschumo	26	73,6	5,83	1,14	+ 4,27	0,46	0,09

TABLE 7. *Differences in height between the different seedling generations of Polygonum bistorta together with their quotients D/m (in heavy types).*

	Artebasch	Tatra	Schachen	Eschumo
Artebasch	×	22,1 ± 2,27 **9,7**	24,9 ± 2,13 **11,7**	46,9 ± 2,12 **22,1**
Tatra	—	×	2,8 ± 1,82 **1,5**	24,8 ± 1,81 **13,7**
Schachen	—	—	×	22,0 ± 1,63 **13,5**
Eschumo	—	—	—	×

TABLE 8. *Differences in earliness between the different seedling generations of Polygonum bistorta together with their quotients D/m (in heavy types).*

	Eschumo	Artebasch	Schachen	Tatra
Eschumo	×	2,54 ± 0,13 **19,5**	3,00 ± 0,14 **21,4**	3,02 ± 0,13 **23,2**
Artebasch	—	×	0,46 ± 0,15 **3,1**	0,48 ± 0,14 **3,4**
Schachen	—	—	×	0,02 ± 0,14 **0,1**
Tatra	—	—	—	×

while no height difference of statistical significance is found between the Tatra and the Schachen series. The alpine type from Eschumo,

Fig. 3. *Polygonum bistorta.* Seedling plants raised from the Artebasch lowland series (to the left), and from the Eschumo alpine series (to the right). Photo 3. 6. 1930.

on the other hand, is considerably lower in stature than any of the rest. This type, as seen from the tables, is also very much earlier than any of the others. The Tatra and the Schachen series show about

the same degree of earliness, while the difference in earliness between these series and the Artebasch series must be considered as sufficiently proved. Fig. 3 gives an idea of the earliness of the Eschumo type, as compared with that of the Artebasch type. At Åkarp the former starts flowering about three weeks earlier than the latter.

IV. DISCUSSION.

From the results recorded above, it is evident that genotypical differences in regard to height and earliness separate the lowland type from the alpine type in the Altaian populations of the species under discussion. As to the existence of subalpine ecotypes in these species in the Altai, no decisive answer can be given at the present, as the material collected on intermediate levels is insufficient. There are, however, strong indications that at least in *Solidago virgaurea* and in *Bupleurum longifolium* subalpine ecotypes do exist in this region. The capacity on the part of these species to push their way into alpine altitudes and flourish in such situations must, in any case, primarily depend on the existence in these populations of biotypes fitted for the building up of alpine ecotypes. Species populations devoid of such a biotype-material lag behind, and the plant becomes increasingly rarer with increasing altitude. The behaviour of *Saxifraga* (*Bergenia*) *crassifolia* L., one of the most characteristic species of the Altai, is a case in point. It grows copiously on lower levels, becomes rarer in higher altitudes, but is found as scattered individuals in sheltered places even up to the tree limit. However, material of this species, collected at respectively 450 and 1700 m. above sea level, and grown side by side in the Åkarp garden, do not reveal any noticeable differences. The very much different behaviour of the species here dealt with, has been described above. They are just as common in the alpine region as in the lowland region. It should be added that in the Altai several other species are found to behave in much the same way, and the underlying cause is most probably the same. *Polygonum alpinum*, to mention one example, is common in the alpine region and grows abundantly also in the lowland region. *Allium schoenoprasum*, one of the most common plants on the lowland, has a second center of distribution in the subalpine region, etc.

A striking feature characteristic of the investigated alpine ecotypes from the Altai, especially those of *Solidago virgaurea* and *Polygonum bistorta*, is the remarkable height attained in the cultures. They sur-

pass in this respect far the alpine ecotype known from Europe of *Solidago* and other species. A clue to the understanding of this peculiarity is furnished by a consideration of the height proportions typical of the Altaian lowland populations, from which these alpine types most probably have become differentiated. A number of such species populations have been dealt with in a previous publication (TURESSON 1930). It is here shown that with regard to aestival plants the climate of the continental East (with a fair supply of summer precipitation) supports types which combine great height with pronounced earliness. Indeed, the lower levels of the Altai have been found to harbour the very extreme type with regard to height. Compared with this climatic region the maritime region of Western Europe supports types which are markedly late and of low growth. Middle and South Europe, again, favour tall but late types, while in North Europe, to judge from Swedish material, low growth combines with pronounced earliness. It seems most probable that the genotypically tall growing alpine ecotypes of *Solidago virgaurea* and *Polygonum bistorta,* occurring in the alpine region of the Altai, carry some of the height factors present in the lowland giant types of the species, and that, as said before, these alpine ecotypes have been locally differentiated from the Altaian lowland population.

The absence in the Alps and in the Carpathians of a special alpine ecotype of the three species investigated, is a most interesting point. The tabulated data on the seedling generations raised from transplant material, collected in these regions, show sufficiently clear that these series differ genotypically from the Altaian alpine series, and that an alpine type similar or analogous to the one inhabiting the Altai, has not been found. The absence of these species in *regio alpina* of the Alps and the Carpathians, or their scattered occurrence in that region when they do find a shelter there, is no doubt due to the absence of the biotypes necessary for the differentiation of an alpine ecotype. The lack of such a biotype-material in the Alps and in the Carpathians also gives an indication of the strength of the selection to which the migrating populations of these species has been subjected. Assuming a migration of these species into Europe from the East (a migration route which does not seem improbable), the successive changes of the biotype-composition of the population, in response to the different climatic belts traversed, have probably reduced the biotype-stock and brought about a different biotype-composition in the periphery of the distribution area of the species. In a previous publication (TURESSON

1925, pp. 226—230) I have tried to explain the irregular distribution of the alpine ecotype of some species in the Alps and in Scandinavia in a similar way. On the basis of this assumption no great difficulty is met with in explaining the presence of a species in the alpine region of the Altai, and the exclusion of that species from the very same region on European mountains.

The differentiation of an alpine ecotype in one district and its subsequent migration in a »ready made» state into another area during climatic epochs favouring its spread, has no doubt been the course followed by a number of species (cf. TURESSON 1927). This, apparently, does not apply to the alpine ecotypes of these species within the regions discussed, due — possibly — to a relatively recent origin in the Altai of these ecotypes. The supposition that similar Alpines in due time should become differentiated on the Middle European mountains, is, however, from what has been stated above, wholly unwarranted. There are certainly no valid grounds for such a belief, as it is seen that the alpine ecotype of *Solidago virgaurea* and of other species are in Europe exclusively found on Scandinavian mountains, although some of these species no doubt have dwelt just as long, or rather for a longer time, on the Middle European mountains. Necessarily, it depends on the biotype-composition of the species population at the particular geographical point if in that spot a differentiation of a specialised ecotype, through combination (or, possibly, through mutation) shall be possible. In species like those here investigated, where the distribution of the biotypes necessary for the constitution of the alpine ecotype apparently is very uneven and unsymmetrical, the appearance of that ecotype also becomes irregular.

It needs hardly be added that the attempt to explain in Lamarckian terms these irregularities in the differentiation and distribution of the alpine ecotype must prove an utter failure. The results here recorded also stress the necessity of studying the genotypical constitution of a species population, before conclusions are made as to the causes of the presence of a species in one situation, and of its absence in another. When, for instance, the fact that the beech climbs higher in the Alps than the oak, while in the North the oak advances much farther than the beech, is explained by differing temperature conditions in North and South during leafing time, we have all reason to feel sceptical. The biotype-composition of the beech population, as well as that of the oak, is most probably quite another in the Alps than in the North, and a genecological study of these portions of the population therefore

becomes necessary, before any answer can be given as to the cause of the distributional differences between the two trees in North and South.

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Although the following lines on the species question have little or nothing to do with the subject just treated, I have here taken the opportunity to add a few words on that pertinent question, since HERIBERT NILSSON, in a recent publication (HERIBERT NILSSON 1930), has delivered a criticism, somewhat polemical in form, against the views held by me on that topic.

The efforts nowadays made to employ genetical data in delimiting species units, do not meet the approval of HERIBERT NILSSON. He writes (l. c. pp. 80—81): »Es ist ganz merkwürdig zu sehen, wie die mendelsche Analyse und die Resultate der Genik benutzt werden, um eine Verschiebung, Umgestaltung und ein Unklarmachen des Speziesbegriffes hervorzubringen, indem man nach einer besseren Abgrenzung als beim linneischen sucht». His own extended, genetical investigations into the genus *Salix*, leads him, however, to the formulation of the following definition of the species (l. c. p. 88): »Spezies ist ein Genotypenkreis (eine Kombinationssphäre), die aber als Population annähernd konstant ist, weil sie bei Kreuzung mit anderen Spezies inkompatibel oder avital reagiert». Owing to a firm, and as I think, well grounded belief that the species of traditional taxonomy, not least those of Linnean rank, represent compounds of very different order and magnitude, and that therefore the attempt to formulate one standard definition of the »species» is impossible, I proposed in 1922 the two species units *ecospecies* and *coenospecies* (TURESSON 1922) and added a third unit, the *agamospecies*, in 1929 (TURESSON 1929). Now, as HERIBERT NILSSON, at frequent intervals, lets me know that these units and terms are valueless, an examination of his arguments does not seem out of place.

A biotype-compound exclusively built up by apomicts, as *Alchemilla vulgaris*, *Antennaria alpina* or *Potentilla argentea*, differs so radically from amphimict-compounds that, in my opinion, it constitutes a special kind of species unit, an agamospecies. The term, according to HERIBERT NILSSON, is »sinnlos» and identical with the terms species or linneon. It is clear, however, that the agamospecies cannot be identical with the species of HERIBERT NILSSON, as defined by him, since apomicts are unable to interbreed. The term linneon,

on the other hand, covers the agamospecies, as well as many other units, since the definition of the linneon simply runs as follows: »a group of individuals which resemble one another more than they do any other individuals (LOTSY 1916, p. 27)». The inadequacy of such a species definition has long been felt, and HERIBERT NILSSON obviously also disapproves of it, when he now proposes a new one. Our different ways in estimating the apomictic species populations, however, find their expression in the methods adopted: I have thought it necessary to raise them to the rank of a special and distinct kind of species, while HERIBERT NILSSON prefers to let them completely disappear from his species definition.

The intraviable and intrafertile biotype groups, found within such amphimict-populations as in *Viola tricolor* L. (viz. *V. arvensis*, *V. alpestris*, etc.), or in *Erophila verna* (L.) E. MEYER (viz. *E. cochleoides, E. violacea-petiolata,* etc.) are the ecospecies, while the large amphimict-population itself constitutes the coenospecies. That characteristic types can be sorted out from the Linnean species is admitted also by HERIBERT NILSSON, but the great difficulty is this (l. c. pp. 89—90): »Was wird aus den Resten der früheren linneischen Species? TURESSON löst diese Frage in der Weise, dass er die frühere linneische Species Coenospecies nennt. Die Fragmente nennt er Ökospecies — — —». This, at the most, should be the net profit with my system in the case of autogamous species, while in regard to allogamous species matters are still worse. Speaking of these species the statement is made (l. c. p. 90): »bei den allogamen Pflanzen deckt sich die Ökospecies mit der linneischen Species. Hier wird jene nur zu einem Echo der Spezies!» Apart from its rhetorical value no apparent reason can be stated for an estimate of this sweeping character, since great many allogamous and Linnean species are found to correspond to the coenospecies and not to the ecospecies. I may mention *Lychnis dioica* L., *Fragaria vesca* L., *Ranunculus aquatilis* L., *Veronica Anagallis aquatica* L., *Valeriana officinalis* L., *Arctium Lappa* L. [1], etc.

In discussing the application of the proposed species scheme to autogamous species, HERIBERT NILSSON expresses strong doubts as to

[1] When the components in the cross *Lappa officinalis* ALL. × *L. tomentosa* (MILL.) LAM. are described by HERIBERT NILSSON in a previous publication (HERIBERT NILSSON 1910, p. 298) as »zwei scharf getrennte Linnésche Arten» the term »Linnean species» is used in an inappropriate sense, since LINNÉ did not distinguish these species. The way of writing *L. officinalis* L. and *L. tomentosa* L., as HERIBERT NILSSON does, is therefore also wrong.

the existence in nature, within such species, of selectioned biotype groups, and refers to the work of NILSSON-EHLE (1911) on the complicated segregation following upon crosses between pure lines in oats and wheat. »Die Variationsserie wird ganz kontinuierlich. Ob hier wirklich eine Selektion dieser Zwischenlinien in der Natur stattfindet, ist ja nicht bekannt, kaum wahrscheinlich (l. c. p. 89)». The conclusion seems rather perplexing, since it is repeatedly found that in autogamous plants, in cereals as in wild plants, different types of the species are confined to climatically and edaphically different localities, just as in the case of allogamous plants. NILSSON-EHLE (1914) gives examples of this kind in cereals, and in wild plants MÜNTZING's (1930) *Galeopsis*-material might be mentioned, as well as TEDIN (1925) and SINSKAJA (1928) on *Camelina* and other cruciferous species.

The treatment of the different chromosome races within a Linnean species as ecospecies is also met with disapproval. HERIBERT NILSSON states his opinion on this question as follows (l. c. p. 89): »Chromosomale Differenzen sind ja während der letzten Jahre bei vielen Arten bekannt worden, ohne dass man aus dieser Ursache ein Zerlegen der Spezies als notwendig angesehen hat, nicht einmal hieran gedacht hat. Diese Erscheinung findet man nicht nur bei experimentell erzeugten polyploiden Formen, sondern auch bei verschiedenen Genotypen aus einer wildwachsenden Artpopulation, wie z. B. — — —». Some of the species cited by HERIBERT NILSSON, however, are very unfortunately chosen. So for instance *Festuca elatior* and *Festuca ovina* (LEVITSKY and KUZMINA 1927). The former, as is well known, is already split up into a number of species with different chromosome numbers (among them our *F. pratensis* HUDS. with $2n = 14$ and our *F. arundinacea* SCHREB. with $2n = 42$), and the latter species, in the sense of LEVITSKY and KUZMINA, includes so well established species as *F. duriuscula* L., *F. capillata* LAM., *F. sulcata* (HACK.) NYM., and *F. valesiaca* SCHL., some of which differ considerably from *F. ovina* s. str. with regard to chromosome number. When, further, HERIBERT NILSSON does not know that the idea of separating species units within a population, on the basis of differing chromosome numbers, has previously been entertained, it seems timely to remind of the opinion of JÖRGENSEN with regard to the 5- and 10-chromosome races in *Callitriche stagnalis* (JÖRGENSEN 1923, p. 87): »Notwithstanding the fact that the two, in cytological respects so dissimilar types, with regard to the outward morphological characters, are almost identical — the plants with 5 chromosomes have slightly smaller fruits and are of a lighter green

colour than the others — I am inclined to consider them as being of different species. This assumption seems justifiable to me, solely in view of the difference in chromosome numbers between the two types. A difference in chromosome numbers like this is a morphological characteristic of greater import than many purely extrinsic differences, and must absolutely be indicated as something substantial».

HERIBERT NILSSON also objects to the suggestion made by me, that the different chromosome races in *Erophila verna* should be regarded as ecospecies. In my paper of 1929 I quoted the following sentences from WINGE (1926, pp. 332—333): »Möglich ist es, dass die Kleinarten, die sich sozusagen unter unseren Augen bilden, wieder in Gruppen eingeteilt werden können, deren Mitglieder untereinander volle Fruchtbarkeit aufweisen, wogegen Typen, die verschiedenen Gruppen angehören, mangelnde Fertilität zeigen. In solchem Falle muss jede von diesen Gruppen als eine Art betrachtet werden, die eine Reihe reiner Linien (Kleinarten) enthält». HERIBERT NILSSON now answers me with another quotation made from the same paper, where WINGE justly remarks that it »jedenfalls in der Praxis unmöglich und widersinnig sein wird, alle die existierenden konstanten Formen zu benennen und beschreiben». Obviously HERIBERT NILSSON here distorts the facts, since I nowhere have expressed the view that the separate biotypes should be raised to the rank of ecospecies. I have spoken of the biotype groups with different chromosome number, and I have expressed my opinion of these groups (ecospecies) in *Erophila verna* in the following way (TURESSON 1929, p. 328): »Mit unserer Terminologie bilden gerade diese Gruppen, deren Existenz wir nunmehr wohl kaum zu bezweifeln brauchen, ebensoviele Ökospezien, und diese zusammen bauen die grössere Einheit auf, die wir die Coenospezies *Erophila verna* nennen».

HERIBERT NILSSON's sweeping condemnation in his criticism of these questions is in my opinion a little overdone. We are rarely in a position to say that we have solved a problem, and the problem connected with the natural delimitation of species will most probably remain unsolved for a long time yet. Meanwhile every attempt to throw light on that problem, has to be tested on its own merits. Unless I am greatly deceived, the species scheme proposed by me, and already tested with good results in *Galeopsis* (MÜNTZING 1930) and in *Phleum* (GREGOR 1931), will not fall to the ground on such criticism as I have tried to refute above. Far more experimental study would seem to be needed before a fair judgement as to the utility of that scheme can be given.

SUMMARY.

1. Seedling generations, raised from alpine and lowland transplant series of *Solidago virgaurea*, *Bupleurum longifolium* and *Polygonum bistorta* from the East-Altaian mountain range, have been grown together with seedling generations raised from similar transplant series, collected in European mountain regions.

2. An alpine ecotype of these species is found in *regio alpina* of the East-Altaian mountains, differing in regard to height and earliness from the lowland type of the same region, while in the Alps and in the Carpathians no such alpine ecotype has been found.

3. The capacity on the part of these species to push their way into alpine altitudes in the Altai, and to flourish in such situations, depend primarily on the existence in these populations of biotypes fitted for the building up of alpine ecotypes. Species populations devoid of such a biotype-material, here exemplified by the behaviour in the Altai of *Saxifraga* (*Bergenia*) *crassifolia*, lag behind and become increasingly rarer with increasing altitude.

4. The remarkable height attained in the Altai by the alpine ecotype, especially of *Solidago virgaurea* and *Polygonum bistorta*, becomes intelligible on the basis of the assumption that this type has been locally differentiated from the giant types of these species occurring on the Altaian lowland.

5. The absence in the Alps and in the Carpathians of an alpine ecotype, similar or analogous to the one inhabiting the Altai, is no doubt due to the absence in these regions of the biotypes necessary for the differentiation of that type. When the distribution of the biotypes necessary for the constitution of the alpine ecotype is uneven and unsymmetrical, the appearance of that type also becomes irregular.

6. The results recorded stress the necessity of studying the genotypical constitution of species populations, before conclusions are made as to the role played by outer factors in the distribution of a species. The different behaviour of a species in, for instance, the Northern and Southern parts of its distribution area cannot properly be explained unless a genecological analysis is made of these portions of the species population (cf. the behaviour of the beech and the oak in the Alps and in Scandinavia).

7. In the last part of the paper some questions connected with the species problem are discussed, and an anti-criticism of the criticism,

recently delivered by HERIBERT NILSSON (1930) against the views held by the writer on that topic, is given.

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CYTOLOGICAL STUDIES IN ALLIUM

A PRELIMINARY NOTE

BY *ALBERT LEVAN*
LUND

ALTHOUGH the investigations on the cytology of *Allium* begun by me in 1929 are not yet completed, I wish to mention now a few of the results gained in the course of the work. Before long I hope to be able to give a fuller description of my researches, and shall reserve my illustrative material and all more detailed accounts until then.

The number of chromosomes in *Allium* is known for a great many species. Besides the haploid numbers 7, 8, and 16, there has recently been added a new basic number, 9, for *A. karataviense* (TELEŽYŃSKI in TISCHLER 1931), and also within the 8-series a triploid (somatically 24 chromosomes) and hexaploid species (somatically 48 chromosomes) (MESSERI 1930) have been found. Unfortunately, in my earlier paper (1929) I erroneously gave *A. karataviense* the number 8. The correct number is undoubtedly 9, now also controlled in the division in the pollen grain.

A study has now been made of the numbers of chromosomes in some 60 *Allium* forms, some of them already investigated, others new. The numbers found are given in Table 1, the third to fifth columns show whether the chromosomes have been studied in the root tips, the pollen mother cells, or the pollen grains, the last column gives briefly the origin of the material. The names »Lund» and »Copenhagen» imply that the material comes from the Botanical Gardens of Lund and Copenhagen; »Stockholm 1931» means material from Hortus Bergianus, »Stockholm no.» embedded root tips kindly presented by Mr. HANS BURSTRÖM, Experimentalfältet. It has not been possible to make a control determination of all the species, therefore I make a reservation about eventual incorrect determinations. The forms to which the denomination *A. nutans* L. forma (table 1, nos. 20, 37, 38, 44, 55—60) has been given are specially interesting. They are all in good agreement with *A. nutans* L. when determined in accordance with REGEL'S monograph (1875), they are, however, distinguished from each other by small, but quite distinct characters, length, breadth, colour,

TABLE 1. *Numbers of chromosomes in Allium.*

No.	Species	Somatic number	Meiotic number	Number in pollen grain division	Origin
1	*Moly* L.	—	7	7	Lund, Copenhagen
2	*narcissiflorum* VILL.	—	7	7	Copenhagen
3	*ursinum* L.	14	7	7	Lund
4	*albidum*	16	—	—	Stockholm no. 233
5	*amblyophyllum* KAR. et KIR.	16	8	8	Lund
6	*ammophilum* HEUFF.	—	8	—	Copenhagen
7	*angulosum* L.	—	8 + fr.	8, 8 + fr.	Copenhagen
8	*ascalonicum* L.	16	—	—	Lund, Weibullsholm
9	*atroviolaecum*	16	—	—	Stockholm, no. 235
10	*azureum*	16	8	—	Hillegom, Holland
11	*Cepa* L.	16	—	—	Lund, Weibullsholm
12	*fistulosum* L.	16	8	8	Lund, Weibullsholm
13	*flavescens* BESS.	16	—	—	Brno
14	*flavum* L.	—	8	8	Lund
15	*Forminii*	16	—	—	Stockholm no. 234
16	*hymenorhizum* L.	16	8	8	Lund, Copenhagen
17	*Ledebourianum* ROEM. et SCHULT.	—	—	8	Copenhagen, Stockholm 1931
18	*lepidum* v. *Rehmanni*	16	—	—	Stockholm no. 227
19	*leucanthum*	16	—	—	Stockholm no. 237
20	*nutans* L. forma	16	8	8	Lund, »$L_{sub.}$»
21	*obliquum* L.	16	8	8	Lund
22	*Ostrowskianum* RGL.	—	8	8	Lund
23	*paniculatum* L.	—	8	—	Copenhagen
24	*ponticum*	16	—	—	Stockholm no. 240
25	*rubellum*	16	—	—	Stockholm no. 236
26	*sativum* L.	16	—	—	Lund
27	*saxatile* L.	—	8	—	Lund
28	*Schoenoprasum* L.	16	8	8	Lund, Stockholm no. 228
29	*Schoenoprasum* L. forma	—	—	8	Stockholm 1931
30	*Scorodoprasum* L.	16	8	8	Lund
31	*sphærocephalum* L.	—	8	—	Lund
32	*Suworowi* RGL.	—	—	8	Copenhagen
33	*Victorialis* L.	—	8	8	Lund, Stockholm 1931
34	*Yunnanense*	16	—	—	Stockholm no. 217
35	*karataviense* RGL.	—	9	9	Lund
36	*carinatum* L.	24	$^{24}/_{2}$	8—16	Lund
37	*nutans* L. forma	24	$^{24}/_{2}$	8—16	Copenhagen

No.	Species	Somatic number	Meiotic number	Number in pollen grain division	Origin
38	*nutans* L. forma	24	$^{24}/_{2}$	10—30	Lund
39	*amplectens*	—	—	12—14	Copenhagen
40	*macranthum*	28	—	—	Stockholm no. 216
41	*neapolitanum* CYR.	28	—	—	Hillegom, Holland
42	*validum* WATS.	—	—	14	Stockholm 1931
43	*coeruleum* PALL.	32	—	—	Lund
44	*nutans* L. forma	—	—	15, 16	Copenhagen, »$L_{mont.}$»
45	*odorum* L.	32	$^{32}/_{2}$	—	Copenhagen
46	*oleraceum* L.	32	$^{32}/_{2}$	13—17	Lund
47	*Porrum* L.	32	$^{32}/_{2}$	16	Lund, Weibullsholm
48	*roseum*	32	—	—	Hillegom, Holland
49	*rotundum*	—	$^{32}/_{2}$	16	Copenhagen
50	*rotundum* v. *scorodoprasioides*	32	—	—	Stockholm no. 230
51	*Schoenoprasum* L. v. *sibiricum*	32	$^{32}/_{2}$	16	TURESSON'S cultures, Åkarp (TURESSON 1931)
52	*sikkinensis*	32	—	—	Stockholm no. 224
53	*tataricum* L.	—	—	14—16	Prague
54	*vineale* L.	32	$^{32}/_{2}$	—	Lund
55	*nutans* L. forma	42	—	36, 40, 42	Lund »L_2»
56	» » »	42	—	20—21	» »L_4»
57	» » »	44	—	21, 22	» »L_5»
58	» » »	48	$^{48}/_{2}$	22—24, 48	» »$L_{m.}$»,»$L_{n.}$»,»$L_{st.}$»
59	» » »	64	—	—	» »L_{1929}»
60	» » »	68	—	34	» »L_7»

and shape of the point of the leaf, the carina of the flower-stalks etc. Up to the present time I have met with diploid, triploid, tetraploid, hexaploid, octoploid and aneuploid plants of *A. nutans* L. forma. Some of them (table 1, nos. 55—60) have grown spontaneously in a bed at the Botanical Garden, Lund, and probably derive their origin from the triploid form (table 1, no. 38) cultivated there. The latter, like the triploid Copenhagen form (table, no. 37) and contrary to the completely sterile triploid *A. carinatum*, is somewhat fertile: on free flowering one or two seeds develop in every inflorescence.

Thus three basic numbers are present in *Allium*, 7, 8, and 9. New numbers for the genus are $2n = 28$, 42, 44, 64, and 68. The forms examined by me are distributed among the different numbers thus:

2n = ...	14	16	18	24	28	32	42	44	48	64	68
Number of forms....	3	31	1	3	4	12	2	1	1	1	1

Somatic chromosomes. — The somatic chromosomes are best studied in smear preparations of pollen grains. The chromosomes in roots can easily be counted and the constrictions in them observed but it is difficult to distinguish the satellites. The most common chromosome form is the V-form with a median to sub-median constriction for the spindle fiber attachment. The majority of the chromosomes in all the species investigated are of this type. Although differences in size have often been established in these chromosomes of the same species, no attempts have been made to classify them according to size as other scientists have managed to do in related genera, such as *Tulipa, Uvularia, Hyacinthus*, etc., the differences in size in *Allium* being too continuous. There is, however, for inst. in a 48 chromosome species, a considerable difference in size between the largest and smallest chromosomes of the idiogram.

In most species there are chromosomes that are more readily identified. This is especially the case with the satellited chromosomes. They differ according to the position of their attachment constriction, i. e. the chromosome section between the attachment constriction and the satellite varies in size in relation to the other chromosome part. In the diploid species *one* pair is often developed as satellited chromosomes. This is the case in *A. ursinum, Moly, amblyophyllum, fistulosum, hymenorhizum, Ledebourianum, obliquum, Schoenoprasum, Suworowi, Victorialis*, and *karataviense*. In *A. ursinum* and *Moly* the distance between the satellite and the attachment constriction is fairly considerable, in for inst. *A. Ledebourianum* and *Schoenoprasum* it is always very short. The satellite also varies in size and appearance. When observing these conditions, great attention must, however, be paid to the fact that the satellite even in the same species may vary considerably in size and appearance, just as the length of the connection thread varies, dependent on the development stage of the chromosome as well as fixing and staining.

Satellited chromosomes are also present in the polyploids. The triploid *nutans* forms (table 1, nos. 37 and 38) have each three. *A. validum*, a tetraploid within the 7-series, shows two satellited chromosomes in the pollen division, which in both size and appearance approach the satellited chromosomes af *A. ursinum*, a diploid species within the 7-series. In the tetraploid Siberian chive variety 4 satellited chromosomes

are present in the root cells, exactly resembling the two satellited chromosomes of the diploid chive. The hexaploid *nutans* type (table no. 58) has 6 chromosomes in the diploid stage with a subterminal constriction but only 2 of them have satellites. Finally certain species have no satellited chromosomes, e. g. *A. narcissiflorum,* a diploid belonging to the 7-series. It has a submedianly constricted pair of chromosomes similar in appearance to the satellited chromosomes of *A. Moly,* but the satellites are lacking. Among species with 16 chromosomes but without satellited chromosomes, *A. nutans* (table no. 20) and *A. Ostrowskianum* may be mentioned.

The largest chromosomes are found in the 14-chromosome *A. ursinum,* both the other species with the same number having decidedly smaller ones. The *A. Schoenoprasum* types (among these are included *A. Ledebourianum*) and *A. azureum* are examples of species with small, short and slender chromosomes. There are gradations between these two extremes. Thus the triploid *A. carinatum* and the tetraploid *A. oleraceum* have chromosomes uniform in size and somewhat larger than the triploid and tetraploid *nutans* forms. Perhaps when we have become thoroughly acquainted with the shape and size of the chromosomes of a still greater number of species, we may find some guidance in these characters for the systematic classification of the genus.

Meiosis. — a) *Diploid forms.* In the genus *Allium* we find a relatively suitable material for studies of the prophase stages of the meiosis. The chromosome pairing in the zygotene is not very easy to observe, as this stage is of short durance and the chromatine is difficult to stain. The pairing takes place parasyndetically. The lengthiest and consequently the most ordinary stage in the preparations is the pachytene showing chromomeres in a double row. This passes over into the strepsitene when the 4 chromatids appear. At this stage and at early diakinesis true chiasmata are seen, with exchange of chromatids between the two chromosomes. The terminalisation of the chiasmata begins, and simultaneously their number decreases considerably. Thus a decrease of the chiasma number from 10—15 down to 2—3 in each geminus can be observed from strepsitene to metaphase. The most common geminus form in the metaphase is the ring type with one or generally 2 crossarms sometimes developed as side rings, usually placed in a horisontal position. Rod-shaped gemini with only one chiasma are also to be met with. In certain cases, for inst. in *A. Schoenoprasum* it can be established that it is generally the satellited chromosomes that assume the shape of a rod. *A. fistulosum* is so far remarkable as all its

8 gemini are usually cross formed, which was figured already by ISHIKAWA (1897). Similar cross shape is seen in gemini among other *Liliaceæ* genera: certain *Tulipa* gemini (NEWTON 1926), in *Fritillaria* (NEWTON and DARLINGTON 1930). Sometimes the pairing is incomplete in certain chromosome pairs, as e. g. in one chromosome pair of the diploid *A. nutans* form (no. 20).

b) *Polyploid forms.* These forms often develop chromosome associations of different kinds in the reduction division. In the triploid species the prophase stages follow NEWTON's and DARLINGTON's *Tulipa* type (1929) with persistent amphitene stage, and not LESLEY's tomato type (1926). The appearance of the fully developed trivalents is somewhat different in the different triploid species. In the *A. nutans* forms interstitial chiasmata are more easily developed than in *A. carinatum*, therefore more complicated trivalent types are present, such as are described by BELLING for triploid *Hyacinthus* (1925, 1929). The most common trivalent forms are: 1) a V with one rod at each arm; 2) chain of three with or without zig-zag arrangement; 3) »ring and rod»; 4) three rods united in one point; 5) »triple arc». A special modification of no. 2) is a V-shaped chromosome with 2 rods in a row joined to the one arm. Sometimes all the chromosomes in a plate are developed as trivalents, forming together 8 elements, but as a rule a number of univalents lie free. Curiously enough a larger number of univalents are found in the Copenhagen form of *A. nutans* than in the Lund form. In the Copenhagen form the appearance of the divisions is characterized by the univalents which tend to remain at the equator during anaphase. The univalents generally divide longitudinally at the first division and assort at random in the second. The mode of union of the *A. carinatum* trivalents is similar to that of the quadrivalents in the related tetraploid *A. oleraceum*.

At anaphase the chromosomes of the trivalents are distributed at random; the most usual distribution is 12 chromosomes to each pole, the rarest is 8 to the one and 16 to the other pole. This has been statistically controlled in chromosome counts in the division of the pollen grain. It should be noted, however, that the triploid Lund *nutans* forms unreduced pollen grains in a predominant percentage. Consequently, the frequency curve of chromosome number in the pollen grains is bimodal, having one larger mode corresponding to 24 and one smaller corresponding to 12 chromosomes. Unlike MESSERI (1931), who reports that the course of meiosis in the triploid *A. Rhiziridium* form examined by her was normal, I have found that, in the triploid species investigated,

irregularities are extremely common. Thus chromosomes and parts of chromosomes often lag in the first division, and develop micronuclei at interkinesis. This is rather characteristic of the Copenhagen *nutans*, where it is not uncommon to find as many as 15 micronuclei at interkinesis. These often remain in the pollen grains in the form of strongly stainable chromatine lumps.

Quadrivalents are often developed in tetraploid species and races, they are for inst. common in *A. Schoenoprasum* v. *sibiricum, oleraceum,* and *vineale,* less common in *A. Porrum* and *rotundum.* The most ordinary types of quadrivalents are rings and chains. They are often very like HÅKANSSON's amphibivalents (1931). One essential difference, however, is that in *Pisum* it is a question of 2 different chromosome pairs, whilst the quadrivalents are supposed to consist of 4 similar chromosomes, a difference, that ought to find expression in the different position of the chiasmata in relation to each other. Differences are to be found in this respect between *Allium* and *Pisum.* The forming of quadrivalents entails irregularities in the chromosome distribution. Elimination of chromosomes also has been observed. In a species with 32 chromosomes, e. g. *A. oleraceum,* there appear in consequence of this, besides pollen grains with 16 chromosomes, a few with 17 chromosomes and many with 15, 14, and even 13 chromosomes. Thus the elimination brings about a marked displacement of the average.

Larger chromosome complexes in the form of chains and rings have also been observed in the *nutans* forms with a high number of chromosomes.

The formation of unreduced pollen grains is not uncommon in *Allium.* The study of this phenomenon is important as it may also throw light upon the biotype formation within certain multiform groups. Within the *A. Schoenoprasum* group there are, for inst., partly types with 16 chromosomes as our Swedish forms, partly the var. *sibiricum* with 32 chromosomes. In order to understand the origin of the latter, it is important to know that the types with 16 chromosomes sometimes form diploid gametes. This has only exceptionally been noticed in the Swedish »Alvar» type »*forma vegetior*», in which I found, last autumn, single diploid pollen grains in the second flowering. The weather was chilly during this second flowering, therefore in this case the formation of diploid pollen grains is probably referable to the modifying influence of external factors, especially as on this form's first flowering no diploid pollen grains could be found,

in spite of examination of many pollen samples, repeated during the course of two years. In the present year I have received a *Schoenoprasum*-like species from Stockholm (table 1, no. 29). On examination certain stamina were shown to contain a very high percentage of diploid pollen grains, 50 per cent and more. Here also there is evidently a question of a modifying influence, as flowers occurred in the same inflorescence without a single diploid grain. By the way, the idea that *A. Schoenoprasum* v. *sibiricum* should be an autopolyploid gains support, partly in the frequent presence of quadrivalents in its reduction division, partly in the existence of 4 satellited chromosomes exactly like the two in the diploid type.

Diploid pollen grains have been met with in a slight degree in several other species, such as *A. karataviense, obliquum,* and *hymenorhizum.* An *A. oleraceum* type grown at the Botanical Garden, Lund, formed a high percentage of unreduced pollen grains last year. This species, however, is only propagated vegetatively, so in this case the formation of unreduced pollen has no possibility of producing higher autopolyploid *oleraceum* types.

In the *Rhiziridium* species *A. nutans*, already mentioned several times, the formation of unreduced pollen grains is very common. This species belongs to the critical species of the taxonomists. Its variability may in a great measure be due to the presence of somewhat fertile triploid forms, with a high number of unreduced pollen grains. In reality, almost all the *A. nutans* forms examined show formation of unreduced pollen. I have the impression that this at least partly is more genetically conditioned than is the case in the previously mentioned species. Single unreduced grains are to be found in the diploid *nutans* type at Lund (table, no. 20) and in the triploid Copenhagen type (table, no. 37). In the triploid Lund form (table, no. 38) the formation of unreduced pollen, on the contrary, is extremely common. Last year were found unreduced and reduced grains in the ratio 3 : 1. In the present year the unreduced grains were, on examination, in a still greater majority. Very varying conditions are prevalent among the *nutans* forms with high chromosome numbers. In one type (table, no. 57) there were last year very few unreduced grains. Only three could be discovered in 6 slides (each slide containing 5/2 smeared stamina). More numerous were the unreduced grains in form no. 56, which in the same number of slides showed 106 unreduced pollen grains. Of the forms with 48 chromosomes L_{st} (no. 57) has, both last and the present year, a pretty high percentage of unreduced pollen grains.

Last year the proportion of the unreduced grains to the reduced in the form L_6, whose somatic number is not as yet known with certainty (it is somewhere about 64), was about 1 to 4. The form that, of all those examined, is richest in unreduced pollen grains is, however, L_2 (no. 55) with its 42 chromosomes. Slide after slide can be gone through without finding more than single reduced grains. Last year the number of reduced grains counted in 16 slides, fixed on three different occasions, did not amount to more than 26. The same high percentage of diploid pollen grains has been shown in L_2 in the present year also.

The unreduced grains are easily recognizable from the reduced. Seen from above their contours are circular while the contours of the reduced grains are crescent. They consist of hemispheres and are formed in dyads corresponding to the reduced grains' tetrads. Their diameter, within the same form, is as great as the reduced grains'. Concerning the mode of formation of the unreduced grains I can only state here that in the *nutans* forms at least, the mechanism for this is to be sought in the homotypic division. The consequence of this is, that the chromosomes at metaphase of the pollen division always appear in pairs, these pairs being the two chromosome halves from the homotypic division, that have not come apart and now both of them are once more divided to give rise to the vegetative and the generative pollen nucleus.

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